SEPARATION PROCESSES IN THE FOOD AND BIOTECHNOLOGY INDUSTRIES

Principles and Applications

Edited by
A. S. GRANDISON and M. J. LEWIS
Department of Food Science and Technology
University of Reading, UK

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Preface

This book concentrates on the more recent methods and techniques for separating food components and products of the biotechnology industry. Each chapter deals with a specific type or area of application and includes information on the basic principles, industrial equipment available, commercial applications and an overview of current research and development.

The introductory chapter gives a brief overview of food composition and properties, and some of the heat and mass transfer considerations in batch and continuous processes. Separations from solids, liquids and gases are briefly discussed. A summary is provided of the more conventional separation techniques such as screening, filtration and centrifugation, and techniques for removing water, such as evaporation, freeze-concentration and dehydration. However, the main emphasis is on separation processes, which have received less attention in textbooks on food-engineering and food-processing operations. It is hoped that this book will complement and supplement many of these excellent texts. Chapter 2 deals with the use of supercritical fluids for extraction processes, with special reference to carbon dioxide. Chapter 3 deals with pressure-activated membrane techniques, and covers the general principles, reviews the applications of reverse osmosis, and serves as an introduction to Chapters 4 and 5, which deal specifically with the principles and applications of ultrafiltration and microfiltration respectively. The separation and recovery of charged particles by ion exchange and electrodialysis is covered in Chapter 6. Chapter 7 discusses innovative separation processes, and reviews some of the methods being actively investigated, some of which are now coming into industrial practice. Much of the emphasis in these chapters is on the separation and recovery of proteins and biologically active ingredients. Chapter 8 is specifically on the methods available for fractionating fat, and covers the upsurge in interest and recent developments in this area. The book concludes with a chapter on solids separation processes, with special reference to particulates. The physical properties which influence the separation are reviewed, together with sieving, screening and air classification. Wet processing methods for extraction are discussed, together with some miscellaneous applications such as dehulling, peeling and cleaning.

Much of the emphasis is on extraction of macromolecules, increasing the added value of foods and recovering valuable components from by-products and fermentation media. Many of the methods discussed are now in commercial practice, whilst others are being vigorously researched.

A. S. Grandison and M. J. Lewis

Contents

Preface xi

1 Separation processes – an overview 1
A. S. Grandison and M. J. Lewis
1.1 Foods – the raw material 1
1.2 Separation techniques 5
1.2.1 Introduction 5
1.2.2 Separations from solids 7
Solid–solid separations 8
Separation from the solid matrix 9
1.2.3 Separations from liquids 10
Liquid–solid separations 10
Immiscible liquids 11
General liquid separation processes 11
1.2.4 Separations from gases and vapours 13
1.3 Water treatment 15
1.4 References 15

2 Supercritical fluid extraction and its application in the food industry 17
D. Steytler
2.1 Introduction 17
2.2 The supercritical fluid state 18
2.2.1 Physical properties of NCF CO_2 20
Density 20
Viscosity 21
Diffusion 22
Volatility (vapour pressure) 23
Chemical properties 23
Biochemical properties 24

2.3 Properties of NCF solutions 24
2.3.1 Solubilities in NCFs 24
General principles 25
Effect of molecular structure 25
Effect of temperature and pressure 28
2.3.2 Theoretical models (equations of state (EOS)) 28
Entrainers 34
2.3.3 Diffusion coefficients 35
2.4 Factors determining the efficiency of NCF extraction 36
2.4.1 Extraction stage 37
Mechanism of extraction 37
The 'free diffusion' model 38
The 'shrinking core' model 38
Solubility 40
Diffusion coefficient 40
Adsorption 40
The role of water 41
2.4.2 Separation stage 42
2.5 Equipment and experimental techniques used in NCF extraction and fractionation 44
2.5.1 Extraction 44
Pilot plants with recirculation 44
Small pilot plant with total loss of CO_2 45
2.5.2 Fractionation 46
Cascades of separation vessels 47
Zosel's 'hot finger' fractionation column 47
2.6 Applications 48
2.6.1 Decaffeination of coffee and tea 49
2.6.2 Seed oil extraction 51
2.6.3 Purification of lecithin 52
2.6.4 Lowering cholesterol levels in foods 53
2.6.5 Fractionation of high-value oils and fats 53
Butterfat 53
Fish oils 54
2.6.6 Extraction of flavours and fragrances 54
2.7 References 57

3 Pressure-activated membrane processes 65
M. J. Lewis
3.1 Introduction 65
3.2 Terminology 66
3.3 Concentration factor and rejection 69
3.4 Membrane characteristics 70
3.5 Permeate rate 71
3.6 Transport phenomena and concentration polarisation 72

3.7 Membrane equipment 75
3.7.1 Membrane configuration 76
3.8 Safety and hygiene considerations 82
3.9 Reverse osmosis applications 86
3.9.1 Introduction 86
3.9.2 Water treatment 87
3.9.3 Milk processing 88
3.9.4 Fruit and vegetable juices 90
3.9.5 Other applications 91
3.10 References 94

4 Ultrafiltration 97
M. J. Lewis
4.1 Introduction 97
4.2 Processing characteristics 98
4.2.1 Rejection or retention factors 98
4.2.2 Yield 101
4.2.3 Average rejection 103
4.2.4 Practical rejection data 104
4.3 Performance of ultrafiltration systems 105
Permeate flux 105
4.3.1 Transport phenomena and concentration polarisation 106
4.3.2 Fouling 111
4.3.3 Factors affecting flux 114
Energy input 114
4.4 Diafiltration 116
Introduction 116
4.4.1 Washing out at constant volume 116
Washing-in 118
4.4.2 Diafiltration applications 119
4.4.3 Protein fractionation 119
4.5 Ultrafiltration applications 120
4.5.1 Dairy applications 121
4.5.2 Oilseed and vegetable proteins 125
4.5.3 Animal products 127
4.5.4 Biotechnology applications 128
Membrane-based bioreactors 128
Enzyme reactors 128
Membrane fermenters 131
Recovery of components and downstream processing 132
4.5.5 Medical applications: serum fractionation 133
4.6 References 134

5 Microfiltration 141
A. S. Grandison and T. J. A. Finnigan
5.1 Introduction 141
5.2 Theory, materials and equipment 141
5.2.1 Membrane configurations and characteristics 142
5.2.2 Performance of microfiltration systems and membrane fouling 146
5.3 Applications in the food and biotechnology industries 148
5.3.1 Food industry 148
5.3.2 Applications for biotechnology 150
5.4 Conclusions 151
5.5 References 152

6 Ion-exchange and electrodialysis 155
A. S. Grandison
6.1 Ion-exchange 155
6.1.1 Theory, materials and equipment 155
Solute/ion-exchanger interactions 155
Ion-exchange groups 158
Ion-exchange materials 158
Capacity 159
Blinding and fouling 159
Elution 159
Ion-exchange columns 160
Mixed bed systems 160
Stirred tanks 160
6.1.2 Applications of ion-exchange in the food and biotechnology industries 160
Softening 161
Demineralisation 161
Decolorisation 163
Protein purification 163
Purification of other compounds 166
6.2 Electrodialysis 166
6.2.1 Theory and equipment 167
6.2.2 Applications of ED in the food and biotechnology industries 169
6.3 References 173

7 Innovative separation methods in bioprocessing 179
J. A. Asenjo and J. B. Chaudhuri
7.1 Introduction 179
7.2 System characteristics 180
7.2.1 Physicochemical basis for separation operations 180
7.2.2 Kinetics and mass transfer 181
7.3 Liquid–liquid extraction: introduction 181
7.3.1 Aqueous two-phase separation 182
7.3.2 Reverse micelle extraction 185

7.3.3 Perfluorocarbon affinity separations 189
7.3.4 Liquid membrane separations 191
7.4 Solid-based separations 195
7.4.1 Adsorption systems: expanded bed adsorption 195
7.4.2 Continuous adsorption recycle extraction 196
7.4.3 Membrane chromatography 199
7.4.4 Chromatographic and adsorption materials 201
7.5 Other developments 202
7.5.1 Electrically enhanced separations 202
7.5.2 Genetic approaches to protein purification 203
7.5.3 Purification of intracellular proteins 203
7.6 References 204

8 Fractionation of fat 207
K. K. Rajah
8.1 Introduction 207
8.1.1 Crystallisation: nuclei formation and crystal growth 209
8.1.2 Polymorphism 210
8.1.3 Quality of edible oils 210
8.2 Dry fractionation 211
8.2.1 Flat-bed vacuum band filter 213
Florentine continuous filter 213
Vacuband batch filter 214
8.2.2 Rotary drum filters 215
8.2.3 Membrane filters 222
Low pressure 222
High pressure 224
Hydrofilter press 225
8.3 Detergent fractionation 232
8.3.1 The Lipofrac process 232
8.3.2 Crystallisation 232
8.4 Solvent fractionation 236
8.5 References 238

9 Solids separation processes 243
M. J. Lewis
9.1 Introduction 243
9.2 Physical properties of solids 244
9.2.1 Classification of powders 246
9.2.2 Particle size and particle size distribution 247
9.2.3 Particle density 250
9.2.4 Forces of adhesion 252
9.2.5 Bulk properties 252
9.2.6 Bulk density and porosity 252
9.2.7 Flowability 254

9.3 Separation of particulates and powders 256
9.3.1 Size reduction 256
9.3.2 Sieving 258
9.4 Air classification 260
9.4.1 Introduction 260
9.4.2 Commercial air classifiers 262
9.4.3 Process characterisation 264
9.4.4 Applications 268
9.4.5 Cereal separations 268
9.4.6 Legumes 270
9.4.7 Other applications 273
9.5 Wet separation processes 273
9.5.1 Protein recovery 274
9.5.2 Soya processing 274
9.5.3 Wheat protein 276
9.5.4 Other applications 277
9.6 Some miscellaneous solids separations 278
9.6.1 Dehulling 278
9.6.2 Peeling 279
9.6.3 Cleaning of raw materials 279
9.6.4 Sorting and grading 281
Colour sorting and grading 281
9.7 References 283

Index 287

Chapter 1

Separation processes – an overview

A. S. GRANDISON and **M. J. LEWIS**, Department of Food Science and Technology, The University of Reading, Whiteknights, PO Box 226, Reading, RG6 6AP

1.1 FOODS - THE RAW MATERIAL

Food and drink play a vital role in all our lives, providing us with the nutrients essential for all our daily activities, including cell maintenance, growth and reproduction. Although foods are commonplace and much taken for granted, their composition and structure are by no means simple. Firstly, all foods are chemical in nature. For most foods the principal component is water and this water plays an important role in the overall behaviour of that food. One of the most important branches of separation is the removal of water, to save transportation costs and improve microbial stability.

The other components can be classified into major components, such as protein, fat or lipid, sugars, starch and fibre. The minor components include the minerals, which are known collectively as ash, vitamins and organic acids. Information on food composition and the amounts of major and minor components can be found in the *Composition of Foods Tables* (Paul and Southgate, 1978). Table 1.1. illustrates just some of the composition data that is available, for a selection of foods.

Food composition tables are useful in that they provide an average composition. However, some of their limitations are illustrated below, taking milk as an example. It should be noted that similar points could be made about most other foods.

Milk is extremely complex in terms of its chemical composition, containing protein, fat, carbohydrate, minerals and vitamins. There are many different proteins, which can be subdivided into the whey proteins, which are in true solution in the aqueous phase, and the caseins, which are in the colloidal form. The fat itself is a complex mixture of triglycerides and, being immiscible with water, is dispersed as small droplets, stabilised by a membrane, within the milk. The vitamins are classified as water or fat soluble, depending on which phase they most associate with. Some of the minerals, such as calcium and phosphorus, partition between the aqueous phase and the colloidal casein and play a major role in the stability of the colloidal dispersion. In addition, there are many other components present in trace amounts, which may affect its delicate flavour

Table 1.1. Composition of foods (weight/100 g)

	Milk	Apple	Peas	Flour	Beef	Cod
water (g)	87.6 (87.8)	85.6	78.5	13.0	74.0	82.1
protein (g)	3.3 (3.2)	0.3	5.8	9.8	20.3	17.4
fat (g)	3.8 (3.9)	tr.	0.4	1.2	4.6	0.7
sugar (g)	4.7 (4.8)	9.2	4.0	1.7	0.0	0.0
starch (g)	0.0 (0.0)	0.4	6.6	78.4	—	—
fibre (g)	0.0 (0.0)	2.4	5.2	3.4	—	
potassium (mg)	150 (140)	120	340	140	350	320
sodium (mg)	50 (55)	2	1	2	61	77
calcium (mg)	120 (115)	4	15	150	7	16
iron (mg)	0.05 (0.06)	0.3	1.9	2.2	2.1	0.3
phosphorus (mg)	95 (92)	16	100	130	180	170
vitamin C (mg)	1.50 (1.0)	15	25	—	—	—
vitamin B1 (mg)	0.04 (0.06)	0.04	0.32	0.33	0.07	0.08
vitamin B6 (mg)	0.04 (0.06)	0.03	0.16	0.15	0.32	0.33
vitamin D (ug)	0.03 (0.03)	—	—	—	tr	tr
vitamin E (mg)	0.10 (0.09)	0.2	tr	tr	0.15	0.44

* flour – household plain

tr – trace

These values are taken from Paul and Southgate (1978). Figures in parentheses are for milk, taken from McCance and Widdowson's *Composition of Foods Tables* (5th edn) (1991), Royal Society of Chemistry, MAFF. There are slight differences between the reported results.

and processing characteristics and nutritional value, such as trace minerals, organic acids and non-protein nitrogen compounds such as peptides, urea and amino acids. Walstra and Jenness (1984) have listed over 60 components present in milk, at levels that can be readily detected. Milk is also potentially a very unstable material. For example the protein can be made to coagulate by a variety of methods, including heating, addition of the enzyme rennet, acid, salts and ethanol. Also the fat globules rise to the surface under the influence of gravity.

Superimposed on this complex composition is the fact that it is subject to wide variation. Milks from different species differ markedly, and many types of milk other than cow's are consumed worldwide, e.g. sheep, goat, buffalo, camel. Within the same species there are large differences between breeds, and even between individual animals in the same herd. In addition to this, and of prime importance to the milk-processing industry, milk from the same animals is subject to wide seasonal variation, reflecting the change in the animals' diet throughout the year, and the stage of lactation. Factors relating to the handling of milk, such as the pH or the amount of dissolved oxygen, are also important to its stability.

Foods may also be contaminated with matter from their production environment, i.e. soil, water and farmyard. For example milk may be contaminated with dirt, straw, antibiotics, growth hormones, heavy metals, or radionuclides.

In chemical terms alone, there is a great deal of scope for separating the components in milk and some examples are listed:

- water removal to produce evaporated or dried products;
- fat separation to produce creams and butter;
- protein separation to produce cheese or protein concentrates;
- calcium removal to improve stability;
- lactose removal, as a specialised ingredient or for low-lactose products;
- removal of components responsible for tainting raw milk or the cooked flavour of heat-treated milk products;
- removal of radionuclides from milk.

In plant products pesticides and herbicides may additionally be present. Some foods, particularly of plant origin, also contain natural toxins, for example oxalic acid in rhubarb, and trypsin inhibitors, phytates and haemagglutinins in many legumes, cyanogenic glycosides in cassava and glucosinolates in rapeseed (Watson, 1987; Jones, 1992). However, the activity of most of these is reduced during normal processing and cooking methods.

Foods also contain active enzyme systems. For example, raw milk contains phosphatase, lipases and proteases, xanthine oxidase and many others. Fruits and vegetables contain polyphenol oxidases and peroxidases, both of which cause colour changes in foods, particularly browning, and lipoxygenases, which produce rancid off-flavours (Nagodawithana and Reed, 1993).

Therefore foods and wastes produced during food processing provide the raw material for extraction of enzymes and other important biochemicals with a range of applications, especially in the food and pharmaceuticals industries. Some examples are listed in Table 1.2. In the biotechnology industry, similar components may be produced by fermentation or enzymatic reactions and require extraction and purification. Perhaps the simplest example is alcohol, produced by a yeast fermentation, where the alcohol concentration that can be produced is limited to about 15 to 20%, as it inhibits further yeast metabolism. Alcohol can be recovered and concentrated by distillation. For low-alcohol or alcohol-free beers and wines, there is a requirement to remove alcohol. Again distillation or membrane techniques can be used.

A wide range of food additives and medical compounds are produced by fermentation; these include many enzymes, such as proteases for milk clotting or detergent cleaners, amino acids such as glutamic acid for monosodium glutamate (MSG) production, aspartic acid and phenylalanine for aspartame, and lysine for nutritional supplements, organic acids such as citric, gluconic and lactic, and hydrocolloids, such as xanthan gum for stabilising or thickening foods, and a wide range of antibiotics and other medicinal compounds.

In most cases it is necessary to purify these materials from dilute raw materials, which often requires sophisticated separation techniques. In fact a large proportion of the activities of the biotechnology industry is concerned with separations of this nature, which is known as downstream processing. In general, the products produced by bio-processing applications are more valuable than food products, and it is economically feasible to apply more complex separation techniques.

Table 1.2. Biochemicals extracted from foods and by products

Source	Product	Application
Papaya	Papain	Meat tenderisation Beer haze removal
Calf stomach	Rennet	Cheesemaking
Barley	Amylase	Glucose syrup production Baking
Pancreas	Insulin	Control of diabetes
Connective tissue	Gelatin	Gelling agent
Egg	Lysozyme	Food preservative
Soybean	Lecithin	Emulsifier
Horseradish	Peroxidase	Diagnostics
Milk	Lactoperoxidase	Antibacterial
Egg	Ovotransferrin	Antibacterial

Most foods also come contaminated with microorganisms, derived from the environment where they are produced, such as soil, water or the farmyard. These will cause food to spoil or decay, or in the case of pathogenic organisms, cause food poisoning, either directly or by producing toxins. Their activity needs to be controlled. Foods can be pasteurised, blanched, sterilised, and irradiated to control such activity. For liquid products microorganisms can also be removed by membrane sterilisation techniques.

However, it is not only the chemical nature of the food that is important; the organisation and structure of components, and hence the physical properties, are vital considerations to the application of separation techniques. For example, the composition of apples as shown in Table 1.1 appears to be relatively simple. However, to fabricate (create) an apple in the laboratory from these components would be technically impossible. Large differences occur between apples in terms of their colour, flavour and texture which are not apparent from composition tables. Similar considerations apply to many other raw materials. Unfortunately for the food processor, nature does not provide materials of uniform chemical or physical properties. Foods have important physical properties, which will influence the separation technique that is to be selected; some of these are listed in Table 1.3. In addition, the structure of both raw materials and processed foods is very varied. They may exist as emulsions or colloids. They may be non-homogeneous on a macroscopic or microscopic scale, possessing fibrous structure and cellular structure, or layered structures such as areas of fat in meat.

Foods are found as solids or liquids, but gas is frequently incorporated. This may be desirable, as in processed foods such as ice cream, bread or carbonated drinks. However, it may be desirable to remove dissolved gases from liquids such as oxygen or cellular gases from fruit and vegetables before certain processing operations.

This brief introduction has aimed to illustrate the diverse nature of foods and related biological materials, and give an insight into their composition and structure. It is this

complexity and diversity which provides the scope and potential for separating selected components from foods.

Table 1.3. Examples of physical properties of foods, and separation processes to which they relate

Physical property	Separation technique
Size, size distribution, shape	Screening, air classification
Density	Centrifugation
Viscosity	Liquid extraction processes
Rheological	Expression
Surface properties	Froth flotation
Thermal properties	Evaporation, drying
Electrical	Electrostatic sorting
Diffusional	Extraction
	Membrane separations
Solubility	Solvent extraction
	Thermal denaturation
Optical	Reflectance (colour) sorting

1.2 SEPARATION TECHNIQUES

1.2.1 Introduction

Separation of one or more components from a complex mixture is a requirement for many operations in the food and biotechnology industries. The components in question range from particulate materials down to small molecules. The separations usually aim to achieve removal of specific components, in order to increase the added value of the products, which may be the residue, the extracted components or both. All separations rely on exploiting differences in physical or chemical properties of the mixture of components. Some of the more common properties involved in separation processes are particle or molecular size and shape, density, solubility and electrostatic charge. These properties are discussed in more detail elsewhere (Mohsenin, 1980, 1984; Lewis, 1990). In some operations, more than one of these properties are involved. However, most of the processes involved are of a physical nature.

Separation from solids or liquids involves the transfer of selected components across the boundary of the food. In many processes another stream or phase is involved, for example in extraction processes. However, this is not always so, for example expression, centrifugation or filtration. In expression, fruit juice or oil is squeezed from the food by application of pressure. In centrifugation, fat can be separated from water due to their density differences, by the application of a centrifugal force. In filtration there is a physical barrier to the transfer of certain components and the liquid is forced through the barrier by pressure, whilst the solids are retained. The resistance to flow will change throughout the filtration process, due to solids build-up. It can be seen that main driving

forces in these applications are pressure and density differences. As for all processes, separation rates are very important and these are affected by the size of the driving forces involved.

In situations where a second phase or stream is involved, mass-transfer considerations become important; these involve the transfer of components within the food to the boundary, the transfer across the boundary and into the bulk of the extraction solvent. It is also important to increase the interfacial area exposed to the solvent. Therefore, size reduction, interfacial phenomena, turbulence and diffusivities all play a role in these processes. In many applications this additional stream is a liquid, either water or an organic solvent; more recently supercritical fluids, such as carbon dioxide, have been investigated (see Chapter 2). However, in hot-air drying the other phase is hot air, which supplies the energy and removes the water. Mass-transfer considerations are important also in some membrane applications and adsorption processes, where the additional stream is a solid. Other examples of driving force are concentration differences and chemical potential, which are involved in these operations (Loncin and Merson, 1979; Gekas, 1992).

In some processes, both heat and mass transfer processes are involved. This is especially so for separation reactions involving a change of phase, such as evaporation or sublimation. Heat is required to cause vaporisation for evaporation, dehydration and distillation processes. Water has a much higher latent heat of vaporisation (2257 kJ/kg) than most other organic solvents. With solid foods the rate of heat transfer through the food may limit the overall process; for example in freeze-drying the process is usually limited by rate of heat transfer through the dry layer.

Separation processes may be batch or continuous. A single separation process, for example a batch extraction, involves the contact of the solvent with the food. Initially concentration gradients are high and the rate of extraction is also high. The extraction rate falls exponentially and eventually an equilibrium state is achieved when the rate becomes zero. The extraction process may be accelerated by size reduction, inducing turbulence and increasing the extraction temperature. Equilibrium is achieved either when all the material has been extracted, in situations where the volume of solvent is well in excess of the solute or when the solvent becomes saturated with the solute, i.e. when the solubility limit has been achieved, when there is an excess of solute over the solvent. However, the attainment of equilibrium may take some considerable time. Batch reactions may operate far away from equilibrium or close to it.

Equilibrium data is important in that it provides information on the best conditions that can be achieved at the prevailing conditions. Equilibrium data is usually determined at fixed conditions of temperature and pressure. Some important types of equilibrium data are:

- solubility data for extraction processes;
- vapour/liquid equilibrium data for fractional distillation;
- partition data for selective extraction processes;
- water sorption data for drying.

Continuous processes may be single- or multiple-stage processes. The stages themselves may be discrete entities, for example a series of stirred tank reactors, or there may

be many stages built into one unit of equipment, for example a distillation column or a screw extractor. The flow of the two streams can either be co-current or counter-current, although counter-current is normally favoured as it tends to give a more uniform driving force over the length of the reactor as well as a higher average driving force over the reactor. In some instances a combination of co-current and counter-current may be used; for example in hot air drying, the initial process is co-current to take advantage of the high initial driving rates, whereas the final drying is counter-current to permit drying to a lower moisture content.

Continuous equipment usually operates under steady state conditions, i.e. the driving force changes over the length of the equipment, but at any particular location it remains constant with time. However, when the equipment is first started, it may take some time to achieve steady-state. During this transition period it is said to be operating under unsteady state conditions. In continuous processes the flow may be either streamline or turbulent. Consideration should be taken of residence times and distribution of residence times within the separation process; the two extremes of behaviour are plug flow, with no distribution of residence times, through to a well-mixed situation, with an infinite distribution of residence times. More detailed analysis of residence time distributions is provided by Levenspiel (1972).

How close the process operates to equilibrium depends upon the operating conditions, flow rates of the two phases, time and temperature. These conditions affect the efficiency of the process, such as the recovery and the size of equipment required.

Finally, all rates of reaction are temperature dependent. Physical processes are no exception, although activation energies are usually much lower than for chemical reaction rates. Using higher temperatures normally increases separation rates.

However, there are limitations with biological materials: higher temperatures increase degradation reactions, causing colour and flavour changes, enzyme inactivation, protein denaturation, loss of functionality and a reduction in nutritional value. Safety issues with respect to microbial growth may also need to be considered.

A brief overview of separation methods is now considered in this chapter, based primarily on the nature of the material or stream subjected to the separation process, i.e. whether it is solid, liquid or gaseous. Other possible classifications are based on unit operations; e.g. filtration, evaporation, dehydration etc. or types of phase contact, such as solid–liquid or gas–liquid contacting processes.

More detailed descriptions of conventional techniques can be found elsewhere – e.g. Brennan *et al.* (1990), Perry and Green (1984), King (1982).

1.2.2 Separations from solids

Most solid foods are particulate in nature, with particles having a large variety of shapes and sizes. Separations involving solids fall into two categories. The first is where it is required to separate or segregate the particles; such processes are classified as solid–solid separations. The second is where the requirement may be to selectively remove one or several components from the solid matrix. Other processes involving solids are concerned with the removal of discrete solid particles from either liquids or gases and vapours (but these will be discussed in other sections).

Solid–solid separations

Separations can be achieved on the basis of particle size from the sorting of large food units down to the molecular level. Shape, and other factors such as electrostatic charge or degree of hydration, may also affect these separations. Screening of materials through perforated beds (e.g. wire mesh or silk screens) produces materials of more uniform particle size. Screening contributes to sorting and grading of many foods, in particular fruits, vegetables and cereals. Cleaning of particulate materials or powders in the dry state can be achieved using screens in two ways. Dedusting is the removal of undersize contaminants from larger particles, e.g. beans or cereals. Scalping is the removal of oversize contaminants from powders or small particulate materials, e.g. sugar, flour. A wide range of geometric designs exists, including flat bed and rotary fixed aperture screens, and numerous variable aperture designs are available (Slade, 1967; Brennan *et al.*, 1990).

Differences in aerodynamic properties can be exploited in the cleaning, sorting and grading of particulate food raw materials (e.g. cereals, peas, nuts, flour) in the dry state. Many designs of aspirator have evolved in which the feed is applied to controlled velocity air streams where separation into two or more fractions is effected. Alternatively, differences in hydrodynamic properties can be used in the sorting of foods such as apples or peas.

A combination of particle size and density may be used to separate solids by settlement. If the solids are suspended in a fluid (liquid or gas), separation may be achieved on the basis that larger, more dense particles will settle more rapidly than smaller, less dense ones. This may be aided by the application of centrifugal force in air classification, as discussed in Chapter 9.

Differences in buoyancy between solid particles is the basis of flotation washing of some foods. For example, heavy debris, such as stones or bruised and rotten fruit, may be removed from sound fruit by fluming the dirty produce over a series of weirs.

Froth flotation depends on the differential wetting of particles. In the case of separating peas from weed seeds, the mixture is immersed in a dilute mineral oil emulsion through which air is blown. The contaminating seeds float on the foam and may be skimmed off. On a smaller scale, the method can be used to separate materials which react selectively with a surfactant, such as heavy metals, from a mixture. Surface active agents, such as proteins and other foam-inducing materials, can be separated in a similar manner. These techniques are commonly used in effluent treatment.

Operations where the outer surface of the food is removed also fall into this category. Examples include dehulling of cereals and legumes and peeling of fruit and vegetables (see Chapter 9). Cereals may be cleaned and sorted on the basis of shape to remove contaminants of similar size. Examples of this are disc and cylinder sorters which employ indentations of particular shape to pick up the corresponding food particles.

A range of equipment is also available to separate food units on the basis of photometric, magnetic and electrostatic properties. Red and green tomatoes, or blackened beans or nuts may be separated automatically on the basis of reflectance properties. Magnetic cleaning is used to remove ferrous metal particles from foods, and thus to protect both the consumer and processing equipment. Electrostatic properties may be

exploited in separating seeds which may be of similar size and shape, or in the cleaning of tea.

More detailed information on solid–solid separations is provided in Chapter 9.

Separation from the solid matrix

Many plant materials contain valuable liquid components such as oils or juices in the cellular structure. These may be separated from the pulped raw material by the use of presses, in a process known as expression. Batch type hydraulic systems or continuous roller, screw or belt systems are available for different applications such as fruit juice, wine and cane sugar production, or extraction of oil from seeds. Expression of fruit juices may be aided by the use of enzymes to improve efficiency of expression and to control the pectin level. Some of the physical properties related to expression processes are discussed by Schwartzberg (1983).

An alternative system to recover components from within a solid matrix is extraction, which relies on the use of differential solubilities for extraction of soluble solids such as sugar from sugar beet, coffee from roasted ground beans, juices from fruit and vegetables and from materials during the manufacture of instant tea. The most common extraction material is hot or superheated water. However, organic solvents are used, e.g. hexane for oil extraction and methylene chloride to extract caffeine from tea and coffee. The use of supercritical fluids such as carbon dioxide is covered in detail in Chapter 2. Extraction processes as equilibrium stage processes are covered in more detail by Brennan *et al.* (1990), Loncin and Merson (1979), Perry and Green (1984).

Many oil extraction processes employ expression, followed by solvent extraction, to obtain a high recovery of oil. The crude oil is then subjected to a series of refining processes, involving degumming, decolorisation and deodorisation to remove undesirable components.

Water, the most common component of most foods, can be removed from solids by the process of dehydration; in this case thermal energy is required to effect evaporation of the water, and this is usually supplied by hot air. Hot air drying is classified as liquid phase drying and results in shrinkage and case-hardening and loss of some volatiles of foods. Types of drier include overdraught, throughdraught, fluidised bed and pneumatic driers. These are described in more detail by Brennan *et al.* (1990), Mujumdar (1987). Freeze-drying, whereby the food is frozen and then subjected to a vacuum, provides a method which reduces shrinkage, case-hardening and flavour loss. Sublimation occurs during freeze-drying. Here conditions are controlled such that water is removed directly from its solid phase to its vapour phase, without passing through the liquid state. To achieve this, the water vapour pressure must be kept below the triple point pressure (4.6 mm Hg) (Mellor, 1978; Dalgleish, 1990).

The removal of air from fruit and vegetables, prior to heat treatment in sealed containers, is of paramount importance to prevent excessive strain on the seams during the sterilisation and subsequent cooling. This is accomplished by blanching, using steam or hot water. Nutrient losses due to leaching are minimised using steam (Selman, 1987).

1.2.3 Separations from liquids

This section will cover those situations where the separation takes place from a fluid, i.e. a substance which flows when it is subject to a shear stress. An important physical property is the viscosity of the fluid, which is defined as the ratio of the shear stress to shear rate. Viscosity and its measurement is discussed in more detail by Lewis (1990).

Solid components may be present dissolved in the liquid, in a colloidal dispersion or in suspension. For example, milk contains lactose, minerals and whey proteins in true solution, casein and calcium phosphate as a colloidal dispersion and fat globules dispersed in the aqueous phase. There may also be sediment resulting from other contaminants of the milk. The objective of the separation may be to remove any of these components.

Liquid–solid separations

Liquid–solid separation applies to operations where discrete solids are removed from the liquid. There are a number of ways of achieving this and these will be briefly reviewed.

Conventional filtration systems separate suspended particles of solids from liquids on the basis of particle size. The liquid component is passed through a porous membrane or septum which retains the solid material either as a filter cake on the upstream surface, or within its structure, or both. Filter media may be rigid, such as wire mesh or porous ceramics, or flexible, such as woven textiles, and are available in a variety of geometric shapes and pore sizes. In practice, the flow of filtrate may be brought about by gravity, the application of pressure greater than atmospheric upstream of the filter (pressure filtration), applying a vacuum downstream (vacuum filtration) or by means of centrifugal force (centrifugal filtration). The theory and equipment for industrial filtration are fully described by Brennan *et al.* (1990). Applications can be divided into those where a slurry containing large amounts of insoluble solids is separated into a solid cake and a liquid, either of which may be the desired product; alternatively clarification is the removal of small quantities (<2%) of suspended solids from a valuable liquid.

Filtration finds applications throughout the food and biotechnology industries. Sugar juices from cane or beet are filtered to remove high levels of insoluble solids, and are frequently clarified at a later stage. Filtration is employed at various stages during the refining of edible oils. In the brewing industry filtration of mash, yeast recovery after fermentation and clarification of beer are carried out. Filtration is used during the manufacture of numerous other foods, e.g. vinegar, starch and sugar syrups, fruit juices, wine, canning brines. In biotechnology, filtration is carried out to clarify and recover cells from fermentation broths.

More recently, membranes with much smaller pores have been introduced. Microfiltration involves the removal of very fine particles or the separation of microorganisms and sterilisation of fluids (see Chapter 5). Ultrafiltration membranes permit the passage of water and components of low molecular weight in a fluid but reject macromolecules such as protein or starch.

Solids may be separated from liquids on the basis of particle size and density using settlement, or using centrifugation. Settlement is a slow process because it relies on the influence of gravity, but is widely used in water and effluent treatment processes. In

centrifugal classification a suspension of insoluble solids (not more than about 1%) is subjected to cyclic motion in a bowl, which subjects the particles to a centrifugal force, many times in excess of the gravitational force. The more dense solid is retained on the inner surface of the bowl while the liquid is tapped off at the centre. An alternative is to use a filtering centrifuge in which the bowl wall is perforated so the liquid is forced out through the wall. The size of the perforations determines what portion of solids is retained in the bowl. Various designs of centrifuge are available for numerous applications such as removal of solids from dairy fluids, oils, juices, beverages, fermentation broths, or dewatering of sugar crystals and corn starches. Such separations may be carried out on a batchwise basis, although automatic and continuous centrifuges are available.

Solid–liquid separation techniques have been covered in more detail by Purchas and Wakeman (1986) and Brennan *et al.* (1990).

Immiscible liquids

Centrifugation in cylindrical bowls provides the simplest method to separate immiscible liquids of different densities. As the dense and lighter liquid streams are removed throughout, the operation can be carried out on a continuous basis. Either tubular-bowl or disc-bowl type centrifuges are normally used for liquid–liquid separation. The major applications are separating cream from milk, and dewatering oils at various stages during refining.

General liquid separation processes

Extraction of components from liquids can be achieved by contacting the liquid with a solvent which will preferentially absorb the components of interest and can then be separated from the liquid, for example by centrifugation. Such solvent extractions could be used for recovering oils and oil-soluble components of flavour components from liquids. However, such examples are not common in food processing. More information on the development of aqueous two-phase separations is given in Chapter 7.

Other methods of separation involve inducing a phase change within the liquid. Crystallisation methods can be used to separate a liquid material into a solid and a liquid phase of different composition. One or both fractions may be the required product. It is important for subsequent separation of the two phases, that a controlled procedure is adopted to yield uniform crystals of a specified size and shape. Crystallisation can be effected by either cooling or evaporation to form a supersaturated solution in which crystal nuclei formation may or may not occur spontaneously. In many instances it is necessary to seed the solution by addition of solute crystals. Batch and continuous operations are possible, although control of crystal size is much more difficult in continuous systems.

Fat fractionation, resulting from crystallisation of triglycerides of higher molecular weight from a mixture, can be achieved by cooling as described in Chapter 8. Freeze concentration involves the crystallisation of ice from liquid foods such as fruit juices or alcoholic beverages. This has the advantage that concentration can be achieved without the application of heat, although the process is limited by cost, degree of concentration possible and loss of suspended components in the crystalline phase. The freezing process can be achieved in scraped surface heat exchangers. Evaporative crystallisation is used in

the manufacture of salt and sugar. Salt is crystallised from brine in multistage vacuum evaporators, and the crystals are allowed to grow in the circulating brine until they are large enough to settle out of the solution. In sugar crystallisation, the operating temperatures are limited by heat damage to the sucrose, therefore short tube evaporators are normally used. Seeding of sugar syrups is necessary, and the resulting crystals are recovered by centrifugation. Crystallisation can be employed in downstream processing in cases where the product is suitably robust. Citric acid, amino acids and some antibiotics can be crystallised following multistage thermal evaporation.

The other main phase change that can be induced is vaporisation. Removal of the main component, water, from solutions results in volume reduction, which is desirable for minimising storage, packaging and transport costs, and for treatment of effluents. It is often necessary to concentrate prior to operations such as drying and crystallisation. Water removal *per se* can be used as a preservation method when water activity is reduced.

Evaporation is the concentration of a solution by boiling off the solvent, which is usually water. Many designs of evaporator are available, and the choice is largely dependent on the heat sensitivity of the food. Boiling temperature can be lowered by reducing the operating pressure, with most commercial evaporators working in the range 40–90°C. For heat-sensitive materials it is necessary to minimise both temperature and residence time in the heating zone. Energy can be saved by resorting to multiple-effect evaporation and incorporating vapour recompression systems. Evaporation results in a final product which is in the liquid form.

An important part of the evaporation process is the removal of vapour from liquid. Vapour–liquid separations are relatively few in comparison, relying on the large density differences between the vapour and the liquid phase. The high-velocity mixture of liquid and vapour produced in the heat-exchange section (calandria) enters a separate vessel tangentially, the vapour leaves from the top and the liquid from the bottom. Care is taken to ensure that entrainment of liquid in the vapour stream is kept to a minimum. Foam is sometimes a problem in these applications.

Most fluid foods contain volatile flavour and aroma products which are lost during thermal evaporation, which gives rise to a product with inferior flavour. This is particularly applicable to fruit juices. Volatile loss increases with the number of effects in the evaporator, and is likely to be higher for batch processes, where the liquid may pass several times through the heating section. One common solution is to remove the volatile components from the liquid, along with the water vapour and to recover them using a second condenser, operating at a much lower temperature. These volatiles can then be added back to the concentrate.

One special type of evaporation is flash cooling, used to remove unwanted volatile components. This is achieved by heating the liquid, followed by subjecting it to a sudden reduction in pressure, sufficient to cause the fluid to boil. This evaporation process removes some water vapour and volatile components. One example is in removing off-flavours from cream. This process is known as vacreation and has been used to deodorise cream. Another example where flash cooling is built into the process is in direct heating ultra-high-temperature (UHT) processes. The product, such as milk, is preheated to about 75°C in an indirect plate or tubular heat exchanger. It is then contacted with clean steam

in an injection or infusion process. This results in rapid heating of the product up to about 140°C and also about 10–15% dilution. The product is held for 2–4 s to achieve sterilisation, and is then subjected to a flash cooling process, wherein the pressure is suddenly released and the temperature falls almost immediately to about 77–78°C. This causes some of the water to evaporate and this water vapour is separated from the milk. In this way, the solids content of the product is restored to its original value. Flash cooling will also remove both desirable and undesirable flavour components and dissolved oxygen. This is an example of a single-stage equilibrium process.

A recent development involves using steam in a counter-current process to strip off the volatile components in liquids. The contact is achieved in a column in which a series of inverted cones rotate, between a series of stationary cones attached to the wall of the column. The steam is fed into the bottom of the column and the liquid at the top. The arrangement produces thin turbulent films and a large area for mass transfer to take place and incorporates many equilibrium stages in one unit. It has applications for volatile recovery from fruit juices and beverages, production of low-alcohol drinks and removing off-flavours and taints (see Fig. 1.1).

An alternative to evaporation for water removal is reverse osmosis. The method employs membranes that permit the passage of water molecules but are impermeable to solute ions and molecules. Therefore, if a solution is applied to the membranes at a pressure greater than the solution osmotic pressure, water passes through the membrane and solute is concentrated in the feed. This has the advantage that pressure, rather than heat, is the driving force, therefore heat damage is avoided. The theory and equipment for reverse osmosis are described in greater detail in Chapter 3. Reverse osmosis is used extensively for the production of pure water as the permeate, but can also be used for concentrating fluid foods such as milk or fruit juices.

Dehydration is the name given to the process where the resulting product is in the solid form, usually with a moisture content below 10%. Dehydration processes involve the removal of water from solids or liquids. With liquids, preconcentration is an important requisite to reduce capital and energy costs. A whole range of techniques are available such as roller drying, band drying, spray drying and freeze drying, described fully elsewhere (e.g. Brennan *et al.*, 1990). Fluids dried include milk, eggs, coffee, tea, artificial creamers and purees made from fruit and vegetables. Reducing flavour loss and preventing heat-induced colour and flavour changes are important quality aspects.

Dissolved gases can be removed from liquids used in sealed containers by either hot-filling, as near the boiling point as possible or by thermal exhausting boxes, whereby the filled cans are heated by steam or hot water prior to sealing. Hot-filling also reduces the air in the headspace. A process known as steam-flow closing can also be used.

The final method for removing components from liquids involves the use of solid phase, in the form of a resin or beads, i.e. ion exchange. This is covered in more detail in Chapter 6.

Separations from gases and vapours

Filtration may also be used to recover solids suspended in gas. A filter cloth or screen of suitable mesh size is used to retain the solid. Bag filters can be used to recover powders

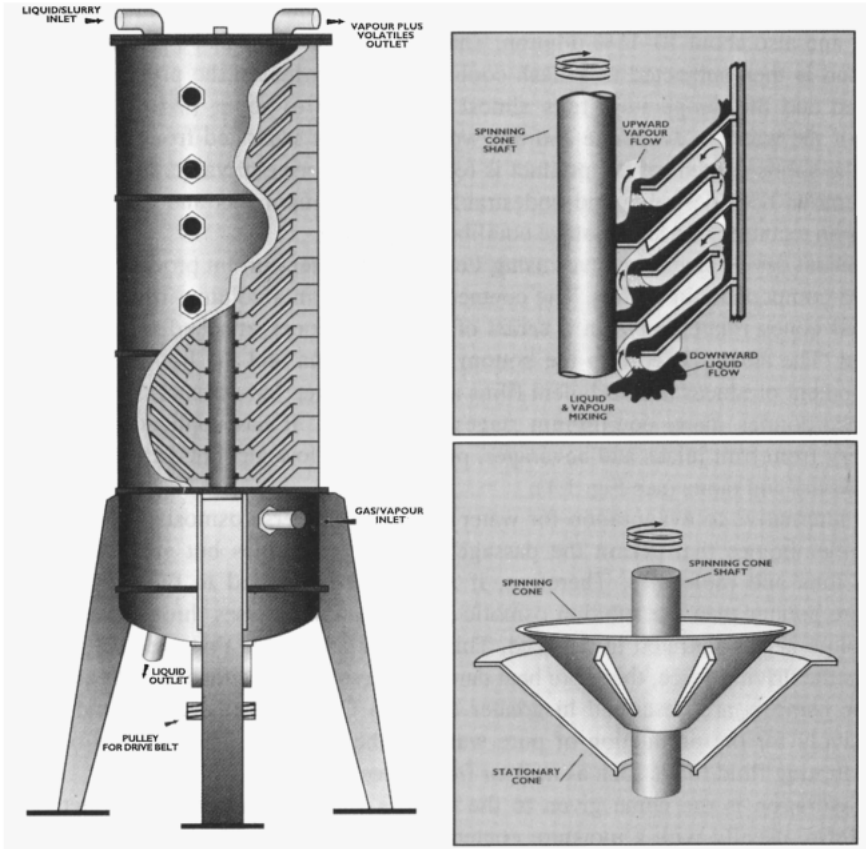

Fig. 1.1. Anatomy of the spinning cone column (by courtesy of Flavourtech).

from air following spray drying, and are frequently used in conjunction with cyclone separators.

Cyclone separators can be used to separate powders from gases on the basis of particle size and density. The solid–gas suspension is introduced tangentially into a cylindrical vessel. The heavier solid particles are thrown to the wall, where on collision they lose kinetic energy and can be collected at the bottom of the vessel, the gas being removed at a separate take-off. Cyclones are employed in powder-handling systems and spray driers.

Wet scrubbing separates suspended solids from gases on the basis of solubility of the solid in a solvent in which the gas is relatively insoluble. Wet scrubbing is used to recover the finest particles from milk drying, by extracting in evaporated milk or water.

The charge on solid particles of suspended solids can be exploited to separate fine solids from gases, by passing the suspension between charged electrodes. The method can be used for recovery of powders, or dust removal from gases.

When potable steam is required for direct steam heating processes, it is important to remove droplets of water, rust and oil. Filtration and centrifugal methods are useful for this purpose.

1.3 WATER TREATMENT

Water is another material which may be required in various levels of purity, depending upon its application. Water purification and the recovery of water from brackish water or sea water (desalination) involves a wide range of separation techniques, but the main process used is fractional distillation. Combinations of conventional filtration and reverse osmosis can also be used to produce potable water from brackish water (see Chapter 4). For more specialised chemical analyses distilled water, double-distilled or deionised water may be required. In the electronics industry there is a high demand for ultrapure water, for the production of microelectronics. The requirements for purity levels increase with the degree of sophistication. The sequence of operations for the production of ultrapure water is illustrated in Fig. 1.2 (Nishimura and Koyama, 1992). Water is subject to RO treatment (twice), conventional filtration, resin treatment to remove anions and cations, degasification, vacuum deaeration, microfiltration and a number of polishing stages.

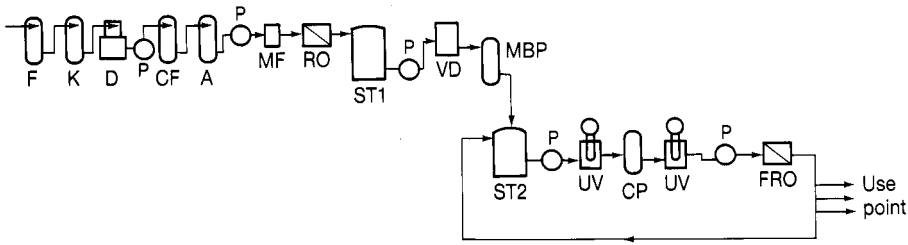

Fig. 1.2. Ultrapure water production system. F, filter; K, cation vessel; D, degasifier; CF, carbon filter; A, anion vessel; MF, micronic filter; RO, intermediate RO; ST1, primary DI water storage tank; VD, vacuum deaerator; MBP, mixed bed polisher; ST2, secondary DI water storage tank; UV, UV steriliser; CP, cartridge polisher; FRO, final RO polisher (from Nishimura and Koyama, 1992, by courtesy of Marcel Dekker).

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Chapter 2

Supercritical fluid extraction and its application in the food industry

DAVID STEYTLER, School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ

2.1 INTRODUCTION

Solvent extraction is one of the oldest methods of separation known and certainly dates back to prehistory. The science of solvent extraction has evolved accordingly over a long period of time and much progress has been made in the understanding of solvation and the properties of liquid mixtures used in extraction processes. The associated literature on phase behaviour is certainly extensive and, although representation of highly non-ideal mixtures is still problematic, many theoretical models have been successfully developed (Fredenslund, 1975; Hildebrand and Scott, 1950; Prausnitz *et al.*, 1986). Extensive databanks of pure component properties have grown to support such models in order to predict solvent performance in process applications. Today, even with the introduction of new separation technologies, solvent extraction remains one of the most widespread techniques operating on an industrial scale. Hannay and Hogarth's (1879) early observations of the dissolution of solutes in supercritical fluid (SCF) media introduced the possibility of a new solvent medium. However, it is only in recent years (since 1960) that commercial process applications of supercritical fluid extraction have been extensively examined.

In the last decade many advances have been made in researching SCF extraction both in terms of fundamental aspects and commercial applications. In particular the high degree of selectivity and control over solubilities afforded by pressure (and temperature) variation has led to the introduction of many novel SCF extraction and fractionation processes. Of all possible gases, the benign properties (non-toxic, non-flammable) and accessible critical temperature of CO_2 have ensured its predominance as a safe SCF solvent for the food industry.

The essential features of a modern solvent extraction process (using a liquid or SCF solvent medium) are illustrated schematically in Fig. 2.1. The material to be extracted is

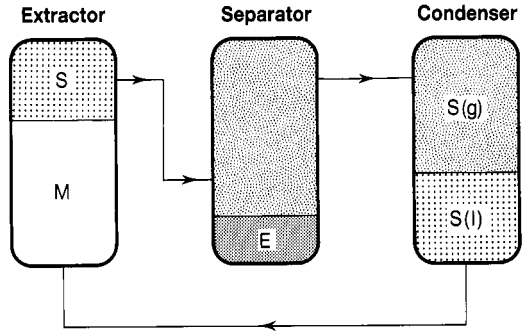


Fig. 2.1. Schematic representation of a solvent extraction process (S = solvent, M = material extracted, E = extract).

placed in an extraction vessel (extractor) into which solvent is introduced under conditions (temperature, flow rate etc.) which optimise the dissolution of the desired components. The solvent stream is then passed to a separation vessel (separator) where conditions are set to selectively separate the solvent from the extracted components. The solvent is then condensed and recycled through the system.

In conventional liquid extraction, solvents of low volatility are employed with vapour pressures less than one atmosphere. In the course of the extraction process the solvent exists as a liquid during the extraction stage and a gas when it is removed from the extract by distillation. Variations in pressure are small and do not significantly exceed the vapour pressure of the liquid at the extraction temperature. Although temperature variation gives some control over solubility, selective removal of components from a mixture is largely determined by the chemical nature of the solvent. Progressive fractionation can therefore only be achieved by a fortuitous response to temperature or by systematically changing the solvent or the composition of a mixed-solvent system.

The initial aims of this chapter are to establish the basic principles involved in SCF extraction. Selected applications are later reviewed with reference to the underlying fundamental properties that serve to differentiate the behaviour of SCFs from conventional liquid solvents.

2.2 THE SUPERCRITICAL FLUID STATE

The P–T phase diagram for CO_2 showing all four physical states (solid, liquid, gas and SCF) is shown in Fig. 2.2. Below the freezing point solid CO_2 ('dry ice') exists which melts on heating when the thermal energy of the molecules overcomes the lattice energy. The integrity of the liquid state so formed is maintained by relatively weak attractive intermolecular forces (van der Waals). The formation of a supercritical fluid state above the critical temperature ($T_c = 31.05°C$) can be viewed as an analogous process in which the thermal energy of the molecules overcomes all attractive interactions maintaining the liquid state. Like a gas, the SCF state formed then occupies all available volume. Strictly the SCF state exists above both the critical temperature and pressure ($T > T_c$; $P > P_c$)

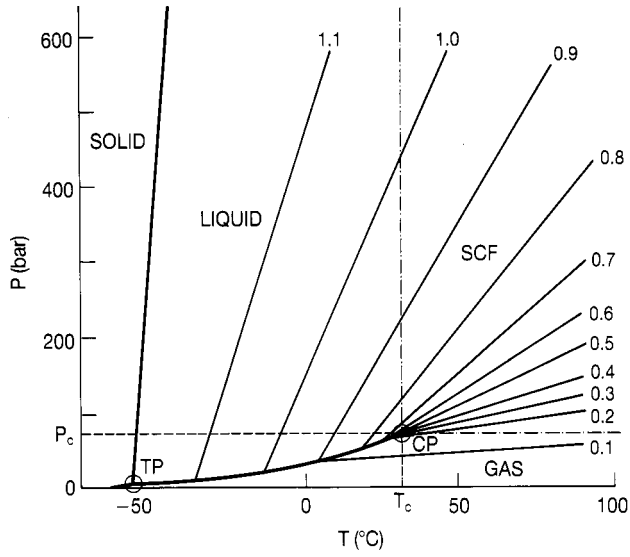


Fig. 2.2. Pressure–temperature phase diagram for CO_2 showing isochores (g cm^{-3}).

though the latter condition is often relaxed in the technical literature. A substance above its critical temperature therefore behaves like a gas and always occupies all available volume as a single phase. However, unlike a gas, a SCF cannot be condensed to a coexisting liquid–gas state by application of pressure. Similarly when the critical pressure is exceeded it is possible to go from a SCF state to a compressed liquid condition by cooling, but a single-phase filling all available volume is always maintained.

It should be appreciated that there are no phase boundaries delineating the SCF state and therefore no sharp changes in physical properties occur on entering this region. Transition to the SCF state from a gas or liquid is thus an 'invisible' process. However, if a coexisting liquid–gas mixture is heated at constant volume along the vapour pressure curve, the density of the liquid phase decreases while that of the gas phase increases, until at the critical point they become equal and the meniscus between them disappears. As this point is approached density fluctuations of microscopic dimensions give rise to a distinctive light-scattering phenomenon known as 'critical opalescence'.

Although the supercritical state offers a greater range of density, which in turn provides greater control over solubilities, the liquid state of compressed gases is often employed in extraction processes, particularly for separation of thermolabile components at low temperatures. In order to avoid restrictive and confusing nomenclature, it is convenient to use the term 'near-critical liquid' (NCL) to distinguish the state of a compressed gas just below T_c from a 'normal' liquid at NTP, for which $T \ll T_c$. The term 'near critical fluid' (NCF) will be used in this chapter to represent both SCF and NCL states of compressed-gas solvents.

Many liquids commonly employed as solvents enter an SCF state on heating, but for most purposes the critical temperatures are too high to permit their use as SCF solvents

(e.g. T_c for hexane is 234°C). All substances with accessible critical temperatures are gases at NTP and representative examples for use in extraction processes are shown in Table 2.1. Being non-toxic, non-flammable, and chemically inert, CO_2 has obvious practical advantages over other potential gases for use in large-scale extraction processes under pressure.

Table 2.1. Potential gases for near-critical fluid extraction

Name	Formula	T_c (°C)	P_c (bar)
Carbon dioxide	CO_2	31.1	73.8
Nitrous oxide	N_2O	36.4	71.5
Ammonia	NH_3	132.4	111.3
Ethane	C_2H_6	32.2	48.2
Propane	C_3H_8	96.6	41.9
Ethylene	C_2H_4	9.2	49.7
Freon 13	$CClF_3$	28.9	38.7

2.2.1 Physical properties of NCF CO_2

Density

Isochores, representing constant density, are shown in Fig. 2.2 for CO_2 in the NCL, gas and SCF regions of the *P–T* phase diagram. In the NCL phase, densities are typical of normal liquid solvents (900–1100 kg m^{-3}) and isothermal compressibility is relatively low. In contrast the SCF state includes a wide range of densities ranging from 'gas-like' values at low pressure (< 100 kg m^{-3}) to 'liquid-like' values at elevated pressure. The region near the critical point is particularly interesting as it represents the region of highest compressibility.

The capability of a solvent to solvate and dissolve a particular solute is directly related to the number of solvent molecules per unit volume. This is because the overall solvation energy is determined by the sum of the solute–solvent interactions occurring primarily within the first solvation shell. Density is therefore a key parameter in determining the effect of temperature and pressure on solubilities in NCF extraction. Indeed, solubility isotherms often exhibit a steep rise with pressure just above the critical point of the solvent where density is rapidly increasing with pressure. The ability to control solubilities through pressure is one of the main features that distinguish NCFs from liquid solvents. Moreover, the potential for differential control of solubilities in multicomponent systems (Johnston *et al.*, 1987) can enable novel fractionation processes that would be impossible using conventional liquid extraction processes.

A systematic assessment of the representation of density, and other thermodynamic properties, of CO_2 by various theoretical models has been made by IUPAC (Angus *et al.*, 1976). This comprehensive treatise provides procedures based on equations of state which

best reproduce available experimental data. An extensive tabulation of properties covering a wide range of pressure and temperature is also included.

Viscosity

Substances that are near their critical temperature at NTP all comprise small, weakly interacting molecules. These characteristics give rise to a high degree of molecular mobility which give NCFs a lower viscosity and higher diffusivity than liquid solvents.

The state of a supercritical fluid approaches that of a gas at low pressure and that of a low-viscosity liquid at elevated pressure. However, to achieve reasonable levels of solubility, the density of the NCF must be modest (> 400 kg m^{-3}). When comparing transport properties with liquid solvents, it is therefore more meaningful to use examples of comparable density. Viscosity isotherms for NCF CO_2 are shown in Fig. 2.3, which includes a density isochore (770 kg m^{-3}) representing typical conditions employed in NCF extraction processes. It is interesting that, at this constant density, the viscosity is not strongly affected by temperature or pressure and has a value of approximately 600 μP. This is to be compared with a typical petrochemical solvent such as hexane, for which $\eta = 3000$ μP at NTP. The smooth transition in physical properties between liquid and SCF states is illustrated by the density isochore which shows no sharp change on passing between the two states. The low viscosity of NCFs and their mixtures can provide some important benefits in extraction processes:

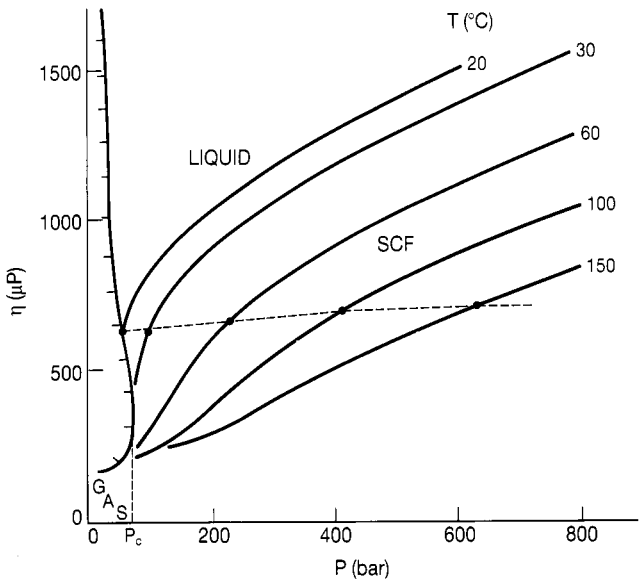


Fig. 2.3. Viscosity isotherms for NCF CO_2 (the dashed line represents a fluid density of 770 kg m^{-3}).

(1) In leaching processes it enables effective percolation of the solvent through packed beds and rapid penetration of the internal pore structure of individual particles comprising the bed.

(2) When a liquid is being extracted the NCF solvent will often dissolve in the coexisting liquid phase and lower its viscosity. This is particularly beneficial when extracting highly viscous liquids which can present mass transfer problems due to poor solvent contact. Indeed NCFs are sometimes used to thin highly viscous materials to facilitate their transport in extraction processes, e.g. the injection of propane into crude lecithin in the NCF separation of phospholipids (Peter, 1987; see Section 2.6.3).

(3) It facilitates transfer of the solvent and reduces the dimensions of pipework required in extraction plants. This is particularly important with high-pressure equipment where costs are strongly linked with scale.

Diffusion

In low-viscosity media, translational diffusion is enhanced and diffusion coefficients in NCFs are therefore significantly higher than in liquid solvents. Self-diffusion isotherms for CO_2 are shown in Fig. 2.4, which also shows an isochore with a typical NCF extraction density of 0.68 g cm^{-3} (680 kg m^{-3}). Again it is found that at constant density the diffusion coefficient is not greatly affected by temperature or pressure and there is no abrupt change on passing between the NCL and SCF states. It is informative to compare

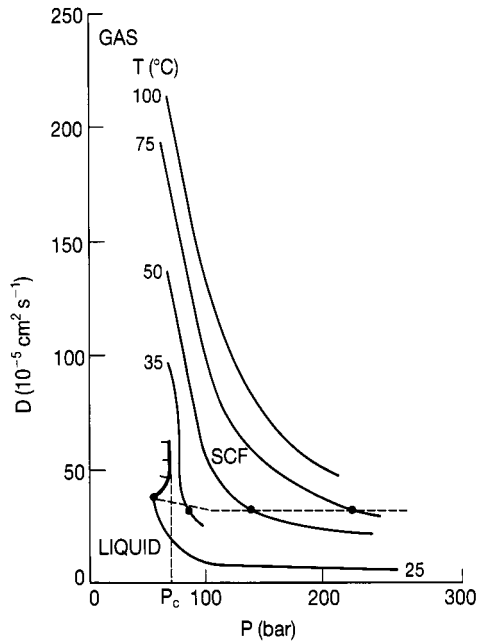


Fig. 2.4. The self-diffusion coefficient of NCF CO_2 (the dashed line represents a fluid density of 680 kg m^{-3}).

the value of the diffusion coefficient under these conditions ($D \simeq 4 \times 10^{-8}$ m^2 s^{-1}) with hexane at NTP ($D = 4 \times 10^{-9}$ m^2 s^{-1}). These values are fairly representative and it is generally observed that self-diffusion coefficients for NCFs under typical extraction conditions are about an order of magnitude greater than in liquid solvents. Diffusion coefficients of solutes in NCFs are generally enhanced to a similar extent (Section 2.3.3).

Volatility (vapour pressure)

In conventional extraction processes liquid solvents are recovered by distillation at elevated temperature (and/or reduced pressure) in which valuable volatile components of the extract can be lost. Near-critical fluids are highly volatile and can be completely removed and recycled at low temperatures during an extraction process. This has important implications for improving the quality of extracts, since:

(1) Highly volatile components in the extract are retained. This is of particular significance in the extraction of flavours and fragrances.
(2) The extract is not subjected to thermal or chemical degradation (e.g. oxidation) at the elevated temperatures employed in distillation.
(3) The high volatility ensures 'complete' removal of solvent residues. Any legislative restrictions regarding residual solvent levels are thereby avoided.

Chemical properties

Of all NCFs, CO_2 is the safest medium for use in solvent extraction as it provides a non-flammable, non-oxidative environment. CO_2 does, however, undergo chemical reactions with water which often need to be considered when extracting food materials.

One familiar set of reactions is the dissolution of CO_2 in water to produce carbonic acid:

$$CO_2 + H_2O \overset{K_1}{\rightleftharpoons} H_2CO_3 \tag{2.1}$$

The carbonic acid then dissociates and lowers the pH of the aqueous phase in contact with CO_2:

$$H_2CO_3 + H_2O \overset{K_2}{\rightleftharpoons} H_3O^+ + HCO_3^- \tag{2.2}$$

$$HCO_3^- + H_2O \overset{K_3}{\rightleftharpoons} H_3O^+ + CO_3^{2-} \tag{2.3}$$

The pH of water is therefore primarily determined by the partial pressure of CO_2 with which it is in contact. Water in contact with atmospheric CO_2 has a pH of approximately 5.7 at 20°C and 3.8 when contacted with pure CO_2 at the same pressure. With increasing pressure the pH falls further, so that when in contact with liquid CO_2 at 100 bar the pH is about 3. This represents a fairly typical acidity for water in an NCF CO_2 extraction process.

Since $K_2 \gg K_3$ the hydrogen ion concentration is primarily determined by the initial dissociation of carbonic acid. If the increased acidity is problematic it is possible to suppress the dissociation and buffer the coexisting aqueous phase by addition of

bicarbonate anion (Lovell, 1988). The pressure of CO_2 could, however, be used to control the pH of water in a unique fashion since no chemical residues (of acids or bases) remain. The potential applications of this technique have not been widely explored.

A less familiar reaction of CO_2 with water is the formation of a solid hydrate below about 10°C:

$$\underset{(g)}{CO_2} + 6\underset{(l)}{H_2O} = \underset{(s)}{CO_2.6H_2O} \qquad (2.4)$$

This restricts the use of NCF CO_2 in the extraction of aqueous systems to temperatures up to 10°C higher than the freezing point of water. (*Note*: This depends on the type and concentration of the solute.)

Biochemical properties

At modest levels CO_2 is non-toxic and so represents a completely safe NCF solvent for food applications with no legislative restrictions governing its use. The only possible, but unlikely, physiological hazard involves asphyxiation by displacement of air following a considerable leak in a confined area.

The combined effects of high hydrostatic pressure and low acidity in water-containing systems can be beneficially employed to prevent food spoilage by destroying bacteria (Kamihira *et al.*, 1987; Taniguchi, 1987a). Rapid decompression of dissolved gas is sometimes used to expand and disrupt the cell structure of natural materials and could also be used as a means of sterilisation. Although SCF CO_2 can be an effective apolar medium for enzyme reactions (van Eijs *et al.*, 1988; Steytler *et al.*, 1991), it has also been used to selectively inactivate enzymes (Taniguchi, 1987b; Weber, 1980). In practice these techniques could be applied either *in situ*, during an extraction process, or as a separate unit operation.

2.3 PROPERTIES OF NCF SOLUTIONS

2.3.1 Solubilities in NCFs

There has been much confusion in some of the literature concerning the solvent properties of NCF CO_2. An impression is often given that NCFs are universal solvents which can be 'tuned' to extract virtually any component of a mixture by selecting a suitable set of conditions of temperature and pressure. Statements to the effect that NCFs are 'good' solvents, implying that solute loadings are high, are also prevalent and highly misleading. Before examining the solvent properties of NCFs in detail, it is worth stating a few basic principles:

(1) To be 'supercritical' intermolecular attractive interactions must be relatively weak compared with thermal energy. This necessitates an absence of all polar interactions, such as hydrogen bonding, and defines a medium of low dielectric constant. All NCFs are therefore essentially apolar solvents.

(2) The absence of strong attractive interactions between molecules means that solvation energies are generally low and solubilities in NCFs are thus often much lower than in liquid solvents.

(3) NCFs can be highly discriminating and frequently offer a greater selectivity than liquid solvents. Any attempt at increasing solubilities by changing conditions or injecting entrainers (see Section 2.3.2) usually serves to reduce selectivity. Although some selectivity is sacrificed it is often preferable to operate at high pressures (and temperatures) to obtain sufficient solubility to make a process viable. The conditions cited for a specific process are often arrived at from an optimisation of these opposing effects of selectivity and solubility. However, the selectivity that is exploited in extraction processes is sometimes an intrinsic property of the NCF solvent and is not always dramatically changed by the conditions (e.g. in the selective extraction of triglycerides from phospholipids; Section 2.6.2).

General principles

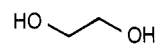
Effect of molecular structure

Any pragmatic assessment of a solvent extraction process must examine what type of molecules are soluble and to what extent. With NCFs the molecular structure of the solute is of major importance as small changes in molecular weight and functional groups can affect solubility to a greater extent than with liquid solvents. In fact the viability of many simple separation processes using NCFs can be realised without recourse to extensive solubility data covering a wide range of conditions. Francis (1954) has painstakingly measured the solubilities of 261 substances in liquid CO_2, and this pioneering work still acts as a useful guide to the relative solubilities of different classes of compounds in NCF CO_2. Dandge *et al.* (1985) have used this and other data to correlate the solubilities of different classes of chemical compounds with molecular structure. Some of the broad principles emerging from this work are given below:

(1) Solubility is reduced by increasing polarity. A good illustration of this is to be found in the relative solubilities of ethanol and ethylene glycol in liquid CO_2. Whereas the former is completely miscible (M), increasing the overall polarity by introducing a second hydroxyl group reduces the solubility to 0.2%. Miscibility with liquid CO_2 can be recovered by methylation of the OH groups, which reduces the polarity of the molecule.

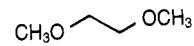
ethanol

(M)



ethylene glycol

(0.2%)



ethylene glycol dimethyl ether

(M)

(2) Solubility declines with increasing molecular weight and for any homologous series the solubility decreases rapidly beyond a given carbon number. This effect is illustrated in Fig. 2.5, which also serves to demonstrate the effect of polarity on solubility since the more polar alcohol has a lower carbon number 'cut off' than the parent alkane.

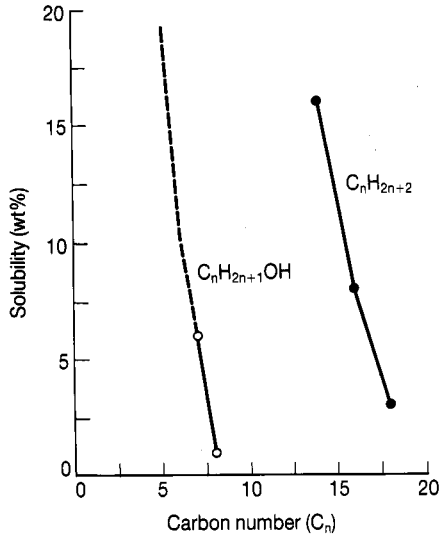
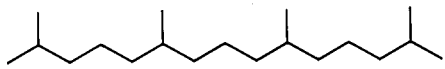
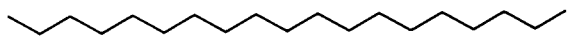


Fig. 2.5. Effect of carbon number (C_n) on the solubility of *n*-alkanes and alcohols in liquid CO_2.

(3) Branching increases solubility. Thus 2,6,10,14 tetramethyl pentadecane is miscible with liquid CO_2, whereas *n*-nonadecane is less than 3% (soluble).

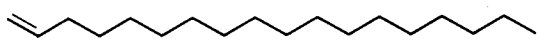


2,6,10,14 tetramethyl pentadecane
(M)

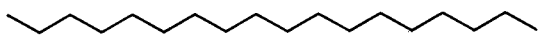


n-nonadecane
(< 3%)

(4) Unsaturation increases solubility as illustrated by 1-octadecene, which is about three times more soluble than its saturated homologue, *n*-octadecane.

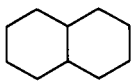


1-Octadecane
(10%)

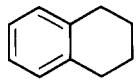


n-Octadecane
(3%)

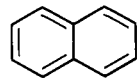
(5) Aromaticity decreases solubility. This is well demonstrated by the progressive decrease in solubility in the series decalin–tetralin–naphthalene as aromaticity is introduced into the molecule:



decalin
(22%)



tetralin
(12%)



naphthalene
(2%)

A summary of some of the solubility characteristics of selected classes of compounds in liquid CO_2 is given below:

(1) Substances with low molecular weight, and low or intermediate polarity are completely miscible.

(a) Aliphatic hydrocarbons (C_nH_{2n+2})

- $n < 12$; (M) (note M = miscible)
- aromatic structures are less soluble but methyl and branched chain substitutions increase solubility.

(b) Alcohols ($C_nH_{2n+1}OH$)

- $n < 6$; (M)
- further hydroxylation reduces solubility.

(c) Carboxylic acids ($C_nH_{2n+1}COOH$)

- $n < 9$; (M)

(d) Esters ($C_nH_{2n+1}COOC_mH_{2m+1}$)

- more soluble than parent acid if $m < n$.

(e) Aldehydes ($C_nH_{2n+1}CHO$)

- $n < 8$; (M)
- aromatic aldehydes are insoluble.

(f) Glycerides. The glycerides illustrate an interesting feature since increasing the extent of esterification of glycerol reduces the polarity but increases the molecular weight. The order of solubility reflects the delicate balance of these opposing effects:

monoglyceride < triglyceride < diglyceride

(2) Macromolecules or highly polar molecules are essentially insoluble, e.g. salts, glycerol, sugars, proteins, starch.

(3) Surfactants. Recently there has been much interest in the formation and properties of reverse micelles and water-in-oil microemulsions in the NCF alkanes (ethane–butane) (Gale *et al.*, 1987; Eastoe, 1990 (a, b)). Moreover, the related 'Winsor II' systems display a clear dependence of droplet size on pressure which could be important in the selective separation of enzymes (McFann and Johnston, 1991). However, although some surfactants are soluble in NCF CO_2, and may well form reverse micelles therein (Consani and Smith, 1990), it is not an effective medium for stabilising microemulsions.

Effect of temperature and pressure

For liquid solvents with low compressibilities the pressure has very little influence on solubility. A simple explanation of the effect of pressure on solubility in NCFs can be made in terms of the number of solute–solvent interactions which depends upon the density of the solvent medium. The overall shapes of solubility isotherms therefore often closely resemble density isotherms of the pure solvent. At very high pressures, restraints of packing can adversely perturb the preferred molecular orientations required for optimum solvation, and solubilities can then begin to decrease with increasing pressure.

As heats of solution are more often positive it is generally observed that solubilities in liquid solvents increase with temperature at constant pressure. However, with NCFs the situation is more complex since both density and temperature must be considered. A general statement governing the influence of these parameters is that 'the solubility increases with increasing temperature at constant density'. This generality is more universally obeyed than the alternative statement in terms of temperature alone.

To illustrate these effects the solubility of naphthalene is shown in Fig. 2.6(a) as a function of temperature and pressure. At constant temperature the solubility increases with pressure in accord with the simple picture of increasing solvation through increasing solvent density. Above about 150 bar the solubility increases with temperature as expected but at lower pressures this 'normal' trend is reversed and the solubility then declines with increasing temperature. This behaviour, which appears anomalous at first sight, can be explained in terms of the high thermal expansivity of the SCF in the lower pressure domain. In this highly expansive region the large drop in density on heating (at constant pressure) outweighs the thermal enhancement and the overall solubility declines. At higher pressures the thermal expansivity of the fluid is much reduced and the solubility then increases with temperature as in liquid solvents. Figure 2.6(b) shows how the solubility dependence can be simplified by replacing the pressure variable with density.

2.3.2 Theoretical models (equations of state (EOS))

Of all theoretical methods used for the prediction of solubilities in NCFs, the solution of phase equilibrium using equations of state has been most widely applied. The appeal of this approach lies in its simplicity, avoidance of intangible standard states and overall success in correlating the phase behaviour of a wide range of NCF mixtures.

To illustrate the general principles involved in the EOS approach, a simple example involving the dissolution of a pure solid (Solute 2) in an NCF (Solvent 1) will be considered as represented schematically in Fig. 2.7(b). Assuming that the NCF does not

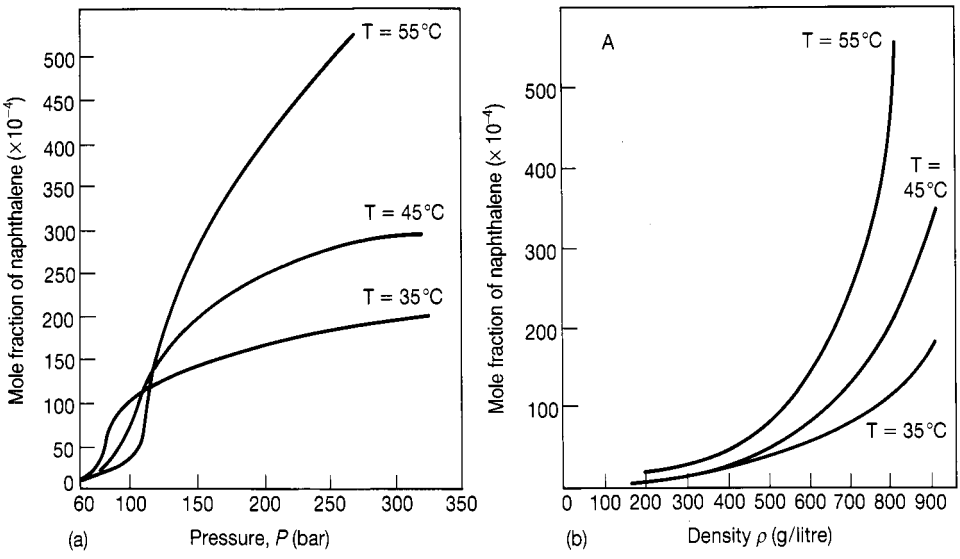


Fig. 2.6. Solubility of naphthalene in NCF CO_2 with (a) pressure and (b) density as variable (reproduced from Brogle, 1982).

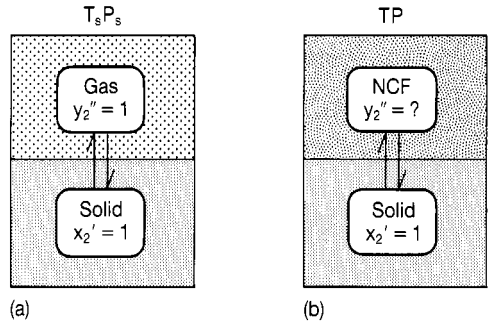


Fig. 2.7. Relationship between (a) sublimation of a pure solid and (b) dissolution in an NCF.

dissolve in the solid phase, the system comprises a pure solid phase, represented by ('), where x = mole fraction of solid in this phase. In this case $x_2 = 1$. This is in equilibrium with an NCF solution represented by ($''$) with an unknown concentration of solid dissolved in it, i.e. $y_2'' = ?$. The NCF phase is often referred to as the 'gas' phase and the symbol y_i accordingly used for mole fraction of component i. The following outlines the procedure for determining y_2''.

The conditions for phase equilibrium are that pressure, temperature and fugacity of each component should be equal in both coexisting phases:

$$T' = T'' = T \tag{2.5}$$

$$P' = P'' = P \tag{2.6}$$

$$f_i' = f_i'' \qquad \text{(for all } i\text{)} \tag{2.7}$$

If it is assumed that the NCF does not dissolve in the solid the last condition is simplified since only the fugacity of the solute (2) need be considered:

$$f_2' = f_2'' \tag{2.8}$$

For an ideal gas mixture the fugacity of each component is equal to the partial pressure:

$$f_i = y_i P \tag{2.9}$$

For general application to any system this relationship is modified to include a non-ideality term, the fugacity coefficient (ϕ_i):

$$f_i = \phi_i y_i P \tag{2.10}$$

The fugacity of the pure solid phase(s) at the system T and P is given by

$$f_2'(T, P) = \phi_2^s P_2^s(T) \tag{2.11}$$

To obtain the fugacity at higher pressure it is necessary to introduce a correction term (the *Poynting correction*):

$$f_2'(T, P) = \phi_2^s P_2^s(T) \exp\left\{\int_{P_2^s}^{P} \left(V_2/RT\right) \mathrm{d}P\right\} \tag{2.12}$$

Poynting correction

where V_2 is the molar volume of the pure solid 2.

If it is assumed that the solid is relatively incompressible, then equation (2.12) can be further simplified:

$$f_2'(T, P) = \phi_2^s P_2^s(T) \exp\left\{V_2\left(P - P_2^s(T)\right)/RT\right\} \tag{2.13}$$

The fugacity of the pure solid phase at the system temperature and pressure can therefore be obtained from sublimation pressure and molar volume data. Using the general form of equation (2.13) to express the fugacity of the solute in the NCF phase and applying the conditions for phase equilibrium ($f_2' = f_2''$),

$$\phi_2^s P_2^s(T) \exp\left\{V_2\left(P - P_2^s(T)\right)/RT\right\} = \phi_2'' y_2'' P \tag{2.14}$$

rearrangement then gives

$$y_2'' = \underbrace{\left(P_2^s(T)/P\right)}_{\text{Perfect gas}} \underbrace{\left(\phi_2^s/\phi_2''\right)}_{\text{Non-ideality}} \underbrace{\exp\left\{\left[V_2\left(P - P_2^s(T)\right)/RT\right]\right\}}_{\text{Poynting correction}} \tag{2.15}$$

Since the vapour in equilibrium with a pure solid phase is usually of low density and can be considered ideal ($\phi_2^s = 1$), equation (2.15) gives the mole fraction of solute in the NCF phase explicitly as a function of its sublimation pressure and molar volume. The only unknown quantity is ϕ_2'', the fugacity coefficient of the solute in an NCF mixture. This can be expressed in terms of the volumetric properties of the mixture as given by equation (2.16), which can be derived from basic thermodynamics (Reid, 1987):

$$\ln \phi_2'' = \frac{1}{RT}\int_0^P \left\{ \left(\frac{\partial V}{\partial n_2} \right)_{T,P,n_2} - \frac{RT}{P} \right\} P \tag{2.16}$$

Solution of equation (2.16) requires an equation of state (EOS) relating the pressure of a mixture to temperature, volume and composition:

$$P = F(T, V, x_i) \tag{2.17}$$

Numerical methods can then be applied to solve the system of equations (2.15)–(2.17) for y_2''.

One of the most familiar equations of state is that of van der Waals (1873):

$$P = \frac{RT}{(V-b)} - \frac{a}{V^2} \tag{2.18}$$

The equation essentially corrects the ideal gas equation ($PV = RT$) for molecular volume (b) and introduces a volume-dependent attractive term (a/V^2). The constants for the pure components (a_i and b_i) are obtained from the critical properties P_c and T_c (values for the constants F_a and F_b are given in Table 2.2).

Table 2.2. Some equations of state

Name	Equation	F_a	F_b
van der Waals	$P = \frac{RT}{(V-b)} - \frac{a}{V^2}$	7/64	1/8
Redlich–Kwong	$P = \frac{RT}{(V-b)} - \frac{a}{T^{1/2}V(V+b)}$	$0.4275\ T^{1/2}$	0.08664
Soave	$P = \frac{RT}{(V-b)} - \frac{a}{T^{1/2}V(V+b)}$	$0.4275\ F(\omega)^*$	0.08664
Peng–Robinson	$P = \frac{RT}{(V-b)} - \frac{a}{V(V+2b)-b^2}$	$0.4572\ F(\omega)^*$	0.07780

* $F(\omega)^* = [1 + (0.3764 + 1.54226\omega - 0.26992\omega^2)(1 - T_r^{1/2})]^2$ where (ω) = the acentric factor representing non-sphericity.

$$a = \frac{F_a R^2 T_c^2}{P_c} \tag{2.19}$$

$$b = \frac{F_b R T_c}{P_c} \tag{2.20}$$

For mixtures, a and b can be evaluated from the pure components values (a_i, b_i) using mixing rules (equations (2.21)–(2.23)):

$$a = \sum_{ij} x_i \, x_j \, a_{ij} \tag{2.21}$$

$$a_{ij} = (a_i \, a_j)^{1/2}(1 - k_{ij}) \tag{2.22}$$

$$b = \sum_{ij} x_i \, b_i \tag{2.23}$$

where k_{ij} = an adjustable interaction parameter.

One important feature of the van der Waals equation is that it can be expressed in an alternative form that is cubic in volume and, given all other parameters, can be solved analytically for V_i and f_i. In the quest to provide improved predictive models for NCF systems the van der Waals equation has provided the starting point for many related cubic equations of state. Two commonly used examples are the Redlich–Kwong (Redlich and Kwong, 1948) and Peng–Robinson (Peng and Robinson, 1976) equations (see Table 2.2) which both introduce a more sophisticated expression for the attraction term but retain the original form of the repulsion term. Both equations can easily be solved analytically or numerically for volume and fugacity coefficient and have been used in numerous correlations of solubility data in NCFs (McHugh and Krukonis, 1986; Prausnitz, 1965; Rizvi *et al.*, 1986). Many variations on the mixing rules (equations (2.21)–(2.23)) have also been proposed (Kwak and Mansoori, 1986; Deiters, 1982; Mathias and Copeman, 1983). A comparative study of the effectiveness of a variety of commonly used equations of state in representing phase equilibrium data in NCF mixtures has been made by Haselow *et al.* (1986).

The simple EOS approach represents the solubilities of volatile, low molecular weight solutes in NCFs reasonably well, as shown by the fit obtained using the Peng–Robinson EOS to the data for naphthalene in SCF CO_2 shown in Fig. 2.8. Inspection of this data reveals how the components of equation (2.15) used in the EOS method contribute to define the solubility curve. In the low-pressure region the behaviour of the NCF is approximated to that of a perfect gas, for which the lowest pressure obtainable is that of the sublimation pressure of the pure solid. The low-pressure limit for the mole fraction of naphthalene in the mixture is simply given by the composition of the mixture at this limiting pressure. As the pressure is increased, y_2'' initially declines in accord with the first 'perfect gas' term in equation (2.15). As the pressure is further increased y_2'' begins to increase as the SCF progressively solvates the solute and ϕ_2'' decreases. Transfer of solute to the SCF phase is also enhanced by the effect of hydrostatic pressure through the Poynting correction term.

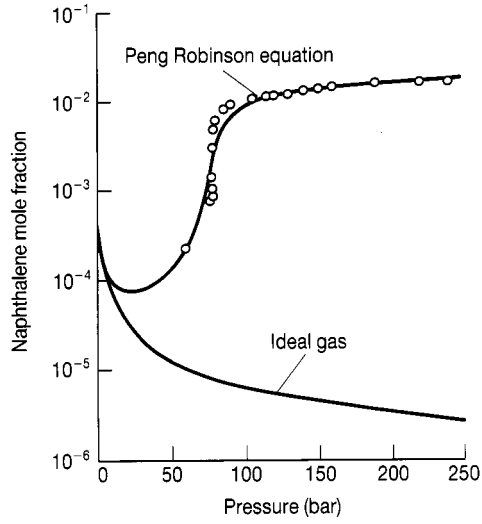


Fig. 2.8. Representation of experimentally determined (○) solubility data for naphthalene in SCF CO_2 at 25°C by the Peng–Robinson equation of state (reproduced from Paulaitis *et al.*, 1983).

The application of the EOS approach to liquid solutes is more involved since the NCF can dissolve in the liquid phase and the fugacities of both components in two coexisting phases must then be considered (Prausnitz and Benson, 1959). Relatively simple computer methods are however available (McHugh and Krakonis, 1986) for an iterative solution of the condition of equal fugacities (equation (2.7)) of each component in both phases. Phase equilibrium data for liquids is usually represented at constant temperature as a function of pressure (or vice versa) as shown in Fig. 2.9 for the butanol/CO_2 system. The region enclosed by the loop represents a two-phase region in which a CO_2-rich 'gas' phase coexists with a butanol-rich 'liquid phase'. At constant pressure the compositions of the coexisting 'liquid' and 'gas' phases are given by the points of intersection of a horizontal 'tie line' within the loop. The relative proportions of the coexisting phases can be obtained in the usual way using the 'lever rule'. In this example, increasing the pressure increases the solubility of butanol in CO_2 until at a critical pressure of approximately 160 bar complete miscibility occurs. This represents the particularly simple behaviour of a liquid with low molecular weight, but as molecular weight and/or polarity is increased the phase behaviour becomes more complex. A rich variety of phase diagrams have been observed and systematically classified for binary NCF mixtures (Schneider, 1970).

One limiting factor which restricts application of EOS models for food-related applications is the lack of available data for the fundamental properties of pure components required for input. Another problem concerns the ambiguity of mixing rules and associated 'adjustable parameter(s)' (e.g. k_{ij} in equation (2.22)) which are often 'floated' in fitting experimental data. Although attempts have been made to define such parameters in terms of pure component properties, EOS models have been more

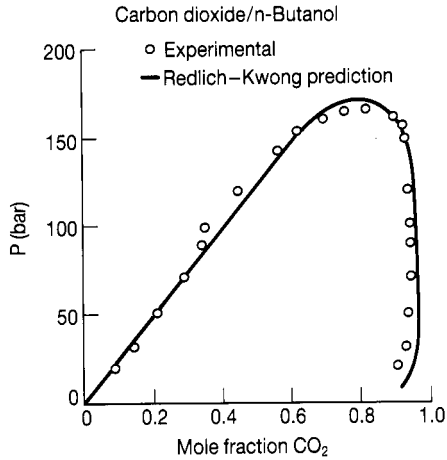


Fig. 2.9. Pressure–composition 'bubble' phase diagram for *n*-butanol/CO_2 at 110°C including theoretical fit by the Redlich–Kwong equation of state (reproduced from King, 1983b).

successful at fitting existing experimental data than in acting in a genuine predictive capacity. This is particularly true for more complex chemical structures of high molecular weight and polarity.

Phase behaviour in NCFs has been of importance in chemical and petrochemical processing for many years and databanks of relevant EOS parameters have grown with the development of these industries. In contrast the stage of development of NCF extraction technology in the food industry is comparatively infantile and data on food-related components is scarce. Some of the large and complex molecular structures found in food systems require more refined methods to correlate their solubilities in NCFs and provide a formidable test for theoretical models. However, many of the components imparting flavour and fragrance comprise small molecules with relatively high vapour/sublimation pressures that can be adequately represented by EOS methods (Brandani *et al.*, de Azevedo *et al.* 1990).

Entrainers

Because solubilities in NCFs are often low, a liquid cosolvent is sometimes added to improve solubility levels of polar, or high molecular weight, substances. Such 'solubility enhancers' are called 'entrainers' (the logic of this nomenclature is unclear – the current Oxford Dictionary definition being 'Put (esp. troops), get, into a train'). Typically the entrainer would be a liquid solvent (e.g. ethanol, acetone, ethyl acetate etc.) which is added at low levels (< 10%) and is completely miscible with the NCF.

Entrainers enhance solubilities in NCFs in much the same way as in mixed solvents systems (Schmitt and Reid, 1986) by introducing stronger molecular interactions in the solvation shell of the solute. In fact there is currently much evidence (Johnson *et al.*, 1990) in support of preferential clustering of the entrainer around the solute molecules such that the solute experiences a local environment that is enriched in entrainer. Various

solvent probes, with ultra-violet/visible (UV/Vis) absorption spectra that are sensitive to the local solvent environment, have been employed to investigate this phenomenon. A familiar food component showing this behaviour is β-carotene which has been the subject of SCF extraction studies (Jay *et al.*, 1991). Figure 2.10(a) shows the local enrichment of the entrainer, ethanol, around β-carotene in SCF CO_2 as determined by UV/Vis spectrophotometry (Jay and Austen, 1990).

Although offering the possibility of improved solubilities there is a tendency to avoid the use of entrainers whenever possible, since they not only reduce selectivity but can also introduce the need for further operations (and associated legislative restrictions), for their removal.

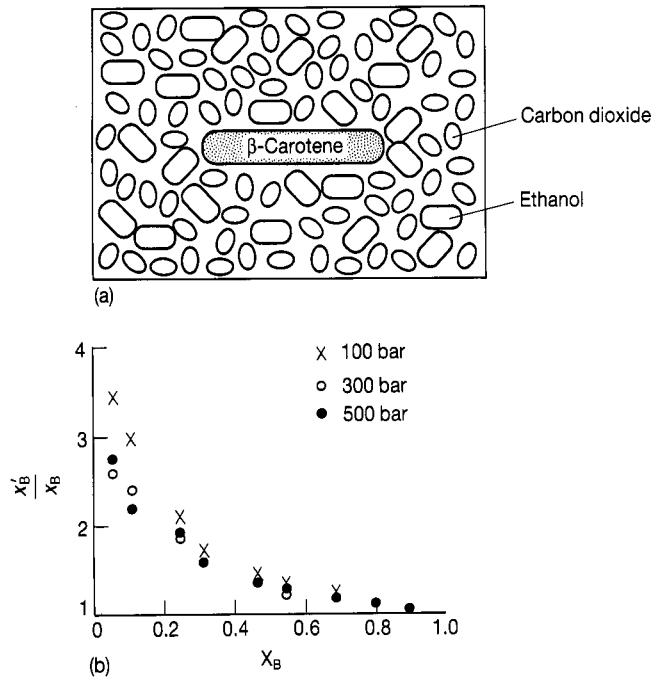


Fig. 2.10. Selective clustering of the entrainer ethanol around β-carotene in SCF CO_2: (a) schematic representation and (b) local enrichment of ethanol given as the mole fraction of ethanol in the solvation shell (x'_β) compared to the solvent mixture composition (x_β). (Reproduced from Jay and Austen, 1990.)

2.3.3 Diffusion coefficients

Diffusion coefficients for solutes in NCF mixtures are significantly higher than in liquid solvents. Typical diffusion coefficient data for a range of solutes are shown in Fig. 2.11 plotted against the density of the NCF solvent. Above a density of about 500 kg m^{-3}, the solute diffusion coefficients are of order 10^{-8} m^2 s^{-1} which is about an order of magnitude greater than corresponding values in conventional liquid solvents.

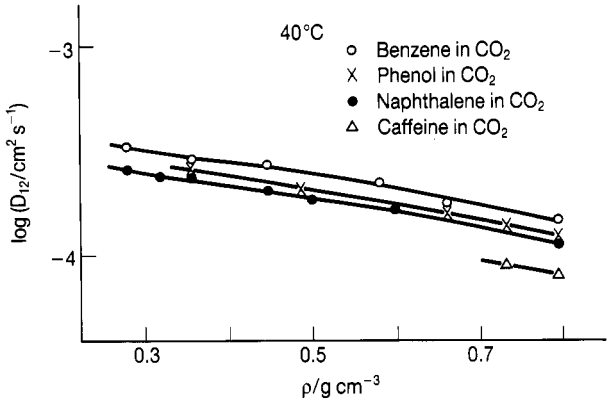


Fig. 2.11. Binary diffusion coefficient (D_{12}) versus solvent density for various solutes in NCF CO_2 at 40°C (reproduced from Feist and Schneider, 1982).

In leaching processes, in which diffusion of solute from a solid matrix is rate-limiting, higher diffusion coefficients could be important in reducing extraction time. Unfortunately this implication is often considered in isolation, which has resulted in the commonly held belief that NCF extraction from porous solid matrices is generally faster than with liquid solvents. This is in fact rarely the case, since a number of other factors (e.g. solubility, adsorption) contribute to the overall rate of elution, so that leaching using NCF extraction is often painfully slow. The interplay between the factors that determine the kinetics of the leaching process is considered further in Section 2.4.

Various models have been employed to correlate diffusion coefficients in NCF systems. In this regard the rough hard-sphere model developed by Chen has been particularly successful in accurately representing both self (Chen, 1983) and tracer (Sun and Chen, 1985) diffusion.

2.4 FACTORS DETERMINING THE EFFICIENCY OF NCF EXTRACTION

In this section the fundamental properties of NCFs that affect their performance as extraction solvents will be examined. Particular emphasis is placed on delineating the differences between SCF and liquid solvents in terms of the physicochemical properties outlined in previous sections. The overall aim is to provide a basis for assessing the viability of a potential extraction process in terms of readily available or measurable parameters.

The efficiency of a solvent extraction process can be judged by both the rate at which the process operates and its energy consumption. The rate-limiting step in the process is most frequently the rate of mass transfer at the extraction stage, which in the case of leaching can be identified as the rate of dissolution of solute from a bed of particles.

The energy input is determined primarily by:

(1) the concentration of solute in the NCF solvent leaving the extraction vessel (which determines the number of cycles required to complete the extraction);
(2) the differential conditions of pressure and temperature between the extraction and separation vessels (which determine the energy consumption per cycle).

In examining process efficiency, the extraction and separation stages will be treated separately.

2.4.1 Extraction stage

To illustrate the differences between NCF and liquids as extraction media the example of leaching of porous particles will be used. This involves the dissolution of a solute, initially evenly distributed throughout the porous matrix within the particles that are themselves insoluble. There are widespread examples of leaching in the food industry, including extraction of seed oils, decaffeination of coffee and isolation of flavour components from plant materials. The rate of removal of solute will depend upon many factors which are common to any solvent, including:

(1) the amount of solute in the particle;
(2) its distribution within the matrix;
(3) the particle size and shape;
(4) the geometry of the porous network.

To simplify the analysis it will be assumed that the solute to be removed is evenly distributed throughout a particle of regular shape (slab) as shown schematically in Fig. 2.12. Similar arguments apply for other geometries (e.g. sphere, cylinder).

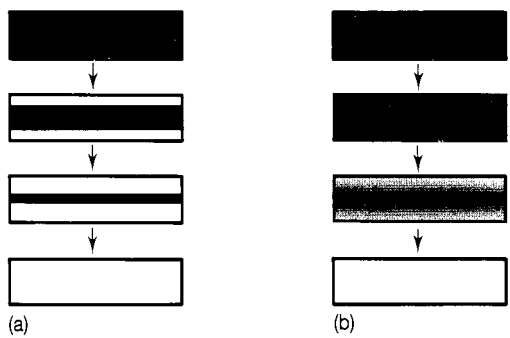


Fig. 2.12. Schematic representation of the extraction of solute from a slab by (a) the 'shrinking core' model and (b) the 'free diffusion' model.

Mechanism of extraction

The solubility of the material to be extracted is clearly a prime factor in determining the effectiveness of a solvent in an extraction process. However, when solubilities are low it can also determine the mechanism by which the extraction proceeds. Because solubilities in SCFs are often low, conditions are frequently incurred which serve to establish a

different mechanism (shrinking core) to that commonly found in liquid solvent extraction (free diffusion).

The 'free diffusion' model

When the solute in the pores is completely dissolved in the solvent it can undergo free translational diffusion in all directions within the confines of the pore network. This condition will be met when the solubility (S) significantly exceeds the concentration (C) of solute within the matrix expressed as the amount of solute per unit volume of solvent in the pores (i.e. the pore volume):

$$S \gg C \tag{2.24}$$

It is more convenient to rewrite this condition expressing C in terms of the overall concentration of solute in the particle (C) and the volume fraction of the pore space (φ)

$$S \gg C/\varphi \tag{2.25}$$

When inequality (2.25) applies, the extraction proceeds by a mechanism in which all of the solute molecules initially dissolve and undergo random translational diffusion, eventually reaching the surface of the particle where they enter the surrounding solvent. The diffusion equations defining the rate of extraction by this mechanism are given by Carman and Haul (1954) for a variety of particle shapes (sphere, cylinder, slab etc.). The fractional extraction of solute (M_t/M_∞) from a infinite slab of half-thickness (L) into a fixed volume of solvent is given below (equation (2.26)):

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-D^{\text{eff}} q_n^2 t / L^2\right) \tag{2.26}$$

where M_t and M_∞ = the mass of solute extracted at time t and infinity, α = the volume ratio of solvent to slab and the q_n terms are the positive non-zero roots of

$$\tan q_n = -\alpha q_n$$

The extraction profile is given by the weighted sum of a series of exponential functions of time. The diffusion coefficient (D^{eff}) in equation (2.26) is the effective diffusion coefficient of the solute in the particle and represents a restricted diffusion process within the pore network. It is related to the bulk diffusion coefficient (D_b) through the tortuosity factor, τ, and the porosity, φ, which accounts for the increased effective diffusion path imposed by the pore structure:

$$D^{\text{eff}} = \varphi D_b / \tau \tag{2.27}$$

A comprehensive treatment of a wide variety of related diffusion problems relevant to solvent extraction is given by Crank (1989).

The 'shrinking core' model

A very different mechanism of extraction applies when the solubility of the solute is low and the condition of inequality (2.25) is reversed, i.e.

$$S \ll C/\varphi \tag{2.28}$$

When inequality (2.28) holds, the solubility limit is reached when only a small portion of the solute within the particle has dissolved. The particle then initially contains a saturated solution of freely diffusing solute in equilibrium with undissolved material evenly distributed throughout the particle. Before more solute can dissolve, the concentration must fall below the solubility limit and this can only happen when solute diffuses from the particle into the surrounding solvent medium. The first solute deposits to be completely eluted are therefore those at the edge of the particle and a boundary of undissolved material is soon established close to the particle surface. As the extraction proceeds this boundary moves progressively towards the centre of the particle and eventually disappears when all solute is dissolved. This mechanism is represented schematically in Fig. 2.12(a) and is often referred to as a 'shrinking core' model. Higuchi (1963), Higuchi and Roseman (1970) have studied the application of this mechanism to the control of drug-release rates from a variety of particle shapes. In the case of a slab of half-thickness (L) the extraction profile is non-exponential and depends upon $t^{0.5}$, as shown in equation (2.29)

$$\frac{M_t}{M_\infty} = \sqrt{\left(\frac{2DSt}{L^2C}\right)} \tag{2.29}$$

Figure 2.13 shows the effectiveness of the 'shrinking core' model in fitting experimental data for which inequality (2.28) applies. The graph represents the SCF CO_2 extraction of β-carotene deposited in a porous slab of sintered bronze (Steytler, unpublished data).

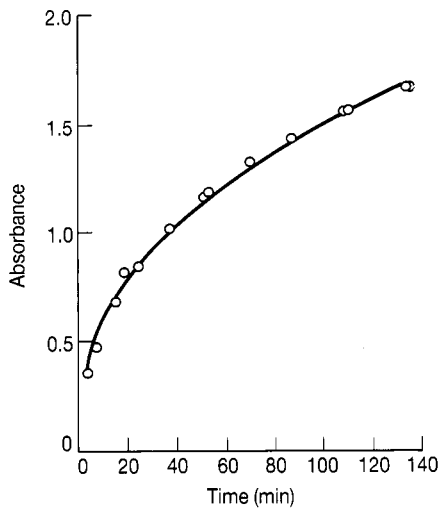


Fig. 2.13. Extraction of β-carotene into supercritical CO_2 (T = 40°C, P = 300 bar) from a porous slab of sintered bronze (L = 0.1 cm). Line shows best fit of eq. (2.29) to the extraction profile using the 'shrinking core' model ($S = 5 \times 10^{-3}$ g dm^{-3}, $C/\phi = 1.9 \times 10^{-1}$ g dm^{-3}).

Solubility

The most important feature of the 'shrinking core' model is that the rate of extraction is determined not only by the diffusion coefficient but also by the solubility of the solute. Clearly when the solubility is low only a small portion of the solute can dissolve and participate in the diffusion process. Rates of extraction can therefore be very low when these conditions apply and this feature has been successfully exploited in the development of devices for controlled drug delivery. Generally solute loadings in SCFs are much lower than in liquid solvents, for which it is possible to choose from a wide range of solvent polarity in order to optimise solubility. The 'shrinking core' mechanism is therefore more likely to prevail in NCF solvents and is often responsible for the slow extraction rates associated with NCF leaching processes.

Diffusion coefficient

It is frequently reported that the higher diffusion coefficients in SCFs give rise to a more rapid extraction than could be achieved using liquid solvents. This is most certainly the case when the 'free diffusion' model applies. Unfortunately the low solubility in SCFs often serves to switch the mechanism to a 'shrinking core' model in which the enhanced diffusion in the SCF is offset by the diminished solubility. Therefore, although having other advantages, SCF extraction can sometimes be very slow in comparison with extraction processes using liquid solvents.

Adsorption

The extent by which a solute molecule in solution partitions to a surface is determined by the differential energy of interaction with the surface (adsorption energy) and solvent (solvation energy). Because solvation energies in SCFs are generally lower than in liquid solvents, partition coefficients to surfaces are often enhanced and adsorption effects are more pronounced. This has significant implications for extraction since adsorption serves both to retard the important rate-determining diffusion processes in leaching and also to reduce the maximum equilibrium concentration of solute that can be attained in the extraction vessel.

In the leaching process the simplest case of rapid adsorption/desorption of solute onto the internal pore surface can be accounted for by a linear isotherm. In this model the diffusion coefficient is replaced by a modified diffusion coefficient (D^{eff}) inversely related to the adsorption coefficient (K_{ads}) (Crank, 1989):

$$D^{\text{eff}} = D/(K_{\text{ads}} + 1) \tag{2.30}$$

where

$$K_{\text{ads}} = \frac{\text{concentration of adsorbed solute}}{\text{concentration of freely diffusing solute}}$$

When $K_{\text{ads}} > 1$ this will clearly reduce the rate of extraction.

In a fixed-bed extraction process, in the absence of adsorption, the maximum attainable concentration of solute in the solvent stream (C^{max}) is given by the solubility. This

determines the minimum amount of solvent required to complete the extraction. In the presence of selective partitioning to the particle bed C^{max} is similarly attenuated:

$$C^{max} = S/(K_{abs} + 1) \tag{2.31}$$

The implications for extraction efficiency are twofold:

(1) More solvent will be required to complete the extraction, i.e. the process must operate through more cycles.
(2) Because the level of the solute in the SCF solvent is lower, a greater pressure differential is required to affect its precipitation in the separation stage of the process.

Both of these factors serve to increase the energy consumption of the process.

The role of water

The addition of entrainers to promote solubility in NCFs has been mentioned previously. In the extraction of plant materials it is often found that the addition of water is essential in order to achieve a realistic extraction rate. The role of water in enhancing NCF extraction is quite distinct from that of entrainers, since it does not usually increase solubilities in the SCF. This is not surprising, since the solubility of water in CO_2 is very low (< 0.5% w/w; Wiebe and Gaddy, 1941). One way in which water affects the rate of extraction is through rehydrating and swelling the internal cellular structure of dried plant material. This can affect the extraction rate in two opposing ways. Firstly increasing the particle size will also increase the distance of diffusion (e.g. L in equations (2.26), (2.29)) within the pore network which will retard the leaching process. A compensating factor, however, is provided by the expansion of the internal structure which can shorten the diffusion path to the surface by opening channels and removing restrictions. Hydrating the plant material can therefore enhance the overall extraction rate.

In some cases water can play a crucial role in determining not only the rate but also the mechanism by which the extraction proceeds. Such behaviour is observed in the decaffeination of tea (or coffee) with SCF CO_2. Dry tea contains approximately 3% w/w caffeine, for which the solubility in SCF CO_2 is between 0.05 and 0.20%. Assuming a pore volume fraction of 0.2 it is clear that the criteria of equation (2.28) are met and in the absence of other factors the extraction of caffeine from dry tea will follow a 'shrinking core' mechanism. The extraction rate is then expected to be strongly solubility-limited. The solubility of caffeine in water, however, is significantly higher (2% w/w at 25°C to 10% w/w at 60°C) than in CO_2. If the tea is completely hydrated, much water is absorbed and the leaves swell to approximately three times their dry volume. All of the caffeine can then dissolve in the water within the internal cellular structure and extraction can proceed by the 'free diffusion' model. These predictions are borne out by experiment (Steytler, 1988) as shown in Fig. 2.14, which represents the extraction of caffeine from the same quantity of wet and dry tea into a fixed volume of SCF CO_2. From the limiting extent of extraction it is clear that caffeine is strongly adsorbed onto the tea and this preferential association persists even when water is added. The selectivity in the extraction of caffeine from wet tea takes place at the water/CO_2 interface at the

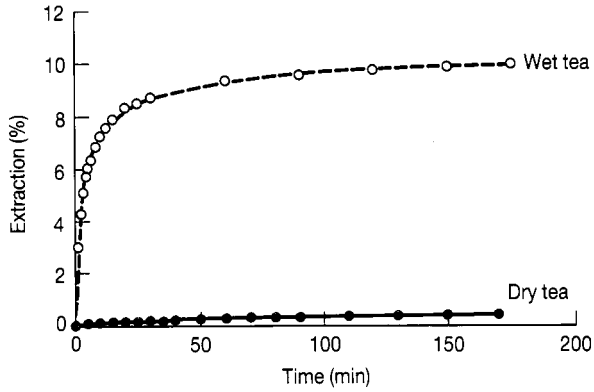


Fig. 2.14. Extraction of caffeine from dry and humidified tea (containing 50% water) into a fixed volume of supercritical CO_2 (T = 40°C, P = 300 bar). The dashed line represents a best fit of the 'free diffusion' model (eq. (2.26)) to the wet tea data.

surface of the leaf. Within the leaf many other water-soluble components may be dissolved and freely diffusing.

2.4.2 Separation stage

Since the solubility of all solutes in SCFs declines with decreasing pressure, the obvious way to separate the SCF at the separation stage is by decompression. This can, however, make heavy demands on energy input to the process, since the SCF then has to be recompressed on each cycle through the system. One solution to this problem has been to preferentially partition the solute to a coexisting solid or liquid phase in the separation vessel maintained at the same pressure as the extractor. This technique is particularly useful for processes in which the extracted components are undesirable contaminants present at low levels. Active carbon can then be employed to selectively adsorb the dissolved components from the CO_2 stream in an 'isobaric' process. In processes for the decaffeination of coffee, caffeine is often removed in this way by adsorption onto active carbon or by partitioning to a coexisting aqueous phase. Unfortunately most low molecular weight organic solvents are miscible with CO_2 (Francis, 1954) and cannot be used for selective partitioning.

A feature of the separation stage, which is currently receiving much attention, is crystallisation. It is generally observed that the rapid decompression accompanying the separation of crystalline solids produces smaller crystals. This has formed the basis of a process for commutation of materials that would otherwise be difficult to handle by conventional milling techniques (Krukonis, 1988). Unlike temperature, pressure variation offers a rapid, isotropic response which can be used to control crystal size (Tavana and Randolph, 1989) and morphology (Ohgaki *et al.*, 1990). One process of relevance to the food industry concerns the application of repeated decompression cycles to induce network growth in aspartame crystals (Steytler *et al.*, 1990) as shown in Fig. 2.15. This

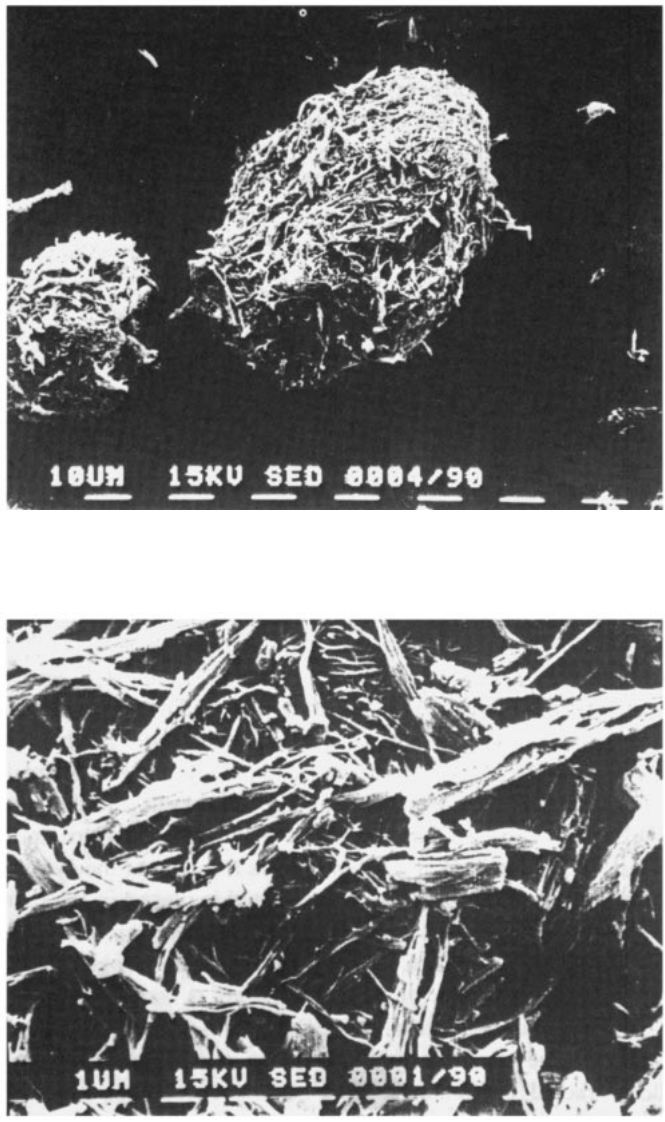


Fig. 2.15. Electron micrographs showing aspartame crystals after repeated decompression cycles in SCF CO_2 at 40°C.

process differs from other techniques of SCF nucleation since it involves repeated dissolution/deposition of material to induce overall particle growth. The product is a free-flowing powder without the 'dust' problems associated with the fine crystalline structure of the starting material.

2.5 EQUIPMENT AND EXPERIMENTAL TECHNIQUES USED IN NCF EXTRACTION AND FRACTIONATION

Although there is some degree of overlap, distinction will be made between equipment that has been designed for use in either fractionation or extraction processes. Details of high-pressure equipment design and engineering are not included here but can be obtained from standard references (Tongue, 1959; Tsiklis, 1968). A description of large-scale commercial-scale plant is also omitted since accurate information of plant operating in the commercial sector is often difficult to obtain and highly process specific. Pertinent design features of large-scale SCF extraction equipment are available in specialist reviews (Bohm *et al.*, 1990; Korner, 1988; Marentis and Vance, 1990; Eggers and Tschiersch, 1980), conference proceedings (Erlangen, 1984) and directly from manufacturers.

2.5.1 Extraction

Pilot plants with recirculation

A typical pilot plant configuration is shown schematically in Fig. 2.16. Although this type of equipment is designed for testing applications on a relatively small scale (< 10 kg) it includes most of the important features of a commercial-scale plant. The essential components of the plant are an extraction vessel, separation vessel, condenser and pump. The CO_2 is stored at its vapour pressure, in the condenser, as an NCL, and is pumped into the extraction vessel by a high-pressure liquid pump. The state of the CO_2 in the extraction/separation stages (SCF/NCL or gas) is determined by the temperature and pressure of the vessels. The temperature is controlled by a thermostatted recirculating fluid flowing through 'heating jackets' surrounding the vessels and the pressure is maintained by pressure relief valves. Conditions of temperature, pressure and flow rate are continuously monitored and logged at strategic points throughout the system.

In an extraction process the material to be extracted is placed in the extraction vessel, which is purged with gaseous CO_2 to remove all air from the system. The extraction is then started by pumping the liquid CO_2 through a heat exchanger into the extraction vessel. The flow rate, which is determined by the pump, is usually set to allow sufficient residence time in the extraction vessel for equilibrium solubility to be attained. The solution then passes to the separation vessel, where conditions are set to minimise the solubility of the extracted components. This often involves decompression to a low-density SCF state ($T > T_c$) or to a state on or below the liquid–gas coexistence line ($T < T_c$). Gaseous CO_2 then passes to the cooled condenser, where it is condensed and stored as a liquid.

Because pressure vessels incorporate thick metal walls, visual monitoring of the extraction process presents some problems. This is particularly pronounced at the separation stage, where it is necessary to monitor the amount and quality of extract obtained during the extraction. If the extract is a liquid it can be periodically removed from the separation vessel by a drain valve, but if a solid it is necessary to halt the extraction to remove the deposits by washing. Non-intrusive monitoring of the extraction can be achieved by spectroscopy (UV/Vis/NIR), if high pressure windows are incorporated in

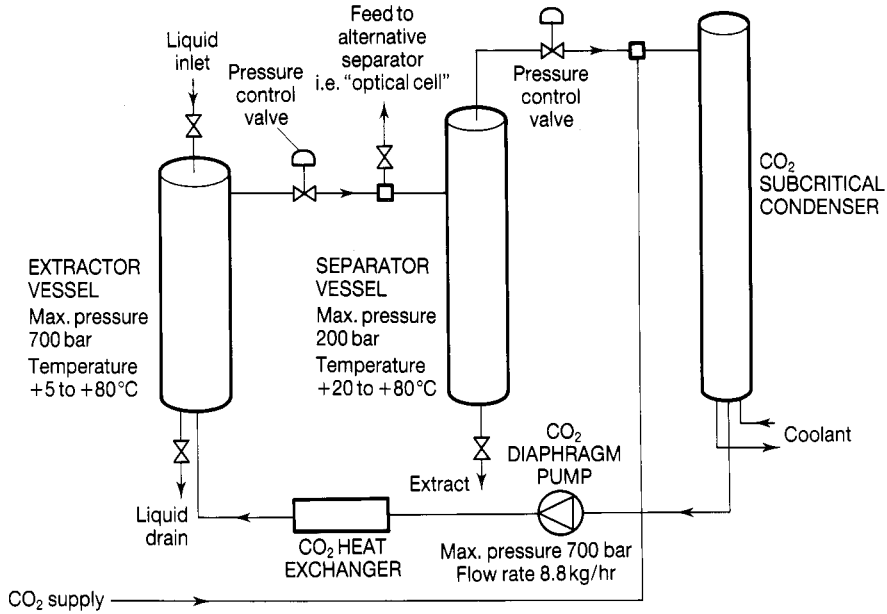


Fig. 2.16. Schematic diagram of an NCF CO_2 pilot plant (capacity 4 dm^3).

the flow line between the extraction and separation vessels. This technique is particularly useful for determining levels of extracted components with chromophoric groups, but is more amenable to optimising established processes where the composition of the extract is known. It is also possible to switch a small sample of the extract stream into an analysis loop for chromatographic identification (GC, SFC, HPLC).

Provision of an additional high-pressure pump for dosing the CO_2 stream with entrainers is a common feature of most pilot plants. In the case of liquid extraction the same pump can be configured to inject the liquid into the extraction vessel in a counter-current mode to establish a continuous process.

Pilot plant operating on this scale (2–10 litres) is useful when it is necessary to extract moderate quantities of material. However, for establishing the feasibility of a process it is often quicker and easier to use smaller-scale equipment.

Small pilot plant with total loss of CO_2

When the scale of extraction is reduced to 10–100 ml, throughput is relatively small and it is no longer necessary to recover and recirculate the CO_2. This allows considerable simplification of the experimental configuration, since at the separation stage the CO_2 stream can be expanded to atmospheric pressure and discarded. A typical configuration is shown in Fig. 2.17.

A small air-driven pump is employed to pressurise the system, and pumps CO_2 directly from a liquid storage cylinder into the extraction vessel. The CO_2 stream is then expanded through a metering valve to atmospheric pressure into a cooled glass Buchner funnel, where the extract is collected. The gaseous CO_2 which is separated then passes

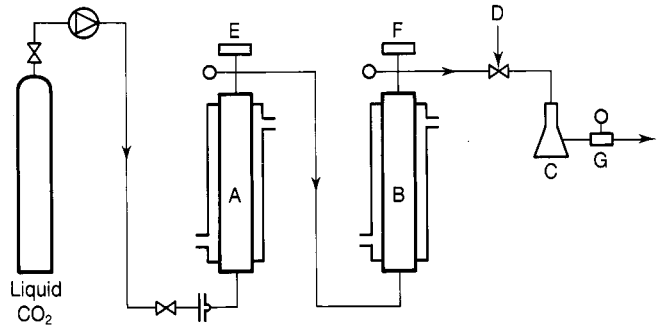


Fig. 2.17. Schematic diagram of a small 'total loss' NCF CO_2 extraction unit: (A) pre-extraction mixing vessel; (B) extraction vessel; (C) extract collection vessel; (D) pressure release-valve; (E, F) thermocouples; (G) flow meter.

through a flowmeter and is discarded. The operation of the system is very simple since the flow rate and pressure are controlled by the metering valve and air-supply pressure to the pump respectively. The only complication with this type of equipment is that considerable cooling accompanies the expansion of CO_2 which must be balanced by heating the expansion valve. If heat input is insufficient, blockages of frozen deposits can produce erratic flow. Conversely, excessive heat input can cause thermal degradation of the extract.

The main advantage of this system is that the extract is always visible and can be removed at any time for analysis by simply changing the receiver. This type of equipment can also be directly interfaced with spectrophotometric and chromatographic analysis.

It is also possible to determine solubilities in CO_2 under various conditions using this equipment (van Leer and Paulaitis, 1980). To study solubility behaviour the substance under examination is placed in the extraction vessel and the flow rate adjusted to ensure that the CO_2 stream becomes saturated. A plot of the weight extracted against throughput of CO_2 then gives the solubility in CO_2 at the pressure and temperature of the extraction vessel.

Total loss systems are valuable research tools for process development studies. They are cheap to install and relatively simple to monitor and operate. There is a growing tendency to reduce the scale of SFE equipment to provide for more rapid screening of potential applications. Miniature extraction systems are now commercially available which are specifically designed to be directly interfaced with chromatographs. Such equipment can be used in screening tests to provide rapid analytical monitoring of the extraction of small quantities of materials.

2.5.2 Fractionation

Although it is possible to fractionate mixtures using the pilot plant equipment described above it is more efficient (in terms of time) to employ equipment specifically designed for this purpose.

Cascades of separation vessels

Fractionation of a mixture can be effected on a 'time-controlled' basis, using the pilot plant described above, by collecting fractions at constant temperature and pressure during the course of the extraction. This is because the more soluble components of the mixture are preferentially extracted in the early stages of the extraction. However, because the solubilities of less soluble components may be very low, this mode of operation can be highly inefficient in terms of time. It is therefore preferable to gradually increase the 'solvent power' of the NCF by progressively changing the conditions of temperature and/or pressure in the extraction vessel. One such procedure involves progressively increasing the extraction pressure in stages such that the more soluble components are removed first. To achieve good separation between stages it would be necessary to continue the extraction at each pressure until equilibrium was approached and the rate of removal of components began to level off. The separation vessel would then have to be emptied, which could involve halting the extraction. This would clearly give rise to a very protracted process.

A simple solution to this problem is to introduce the fractionation at the separation stage of the process by using a cascade of separation vessels as shown in Fig. 2.18. Here the sample is extracted under extreme conditions (high pressure/temperature) at which all extractable components are removed in the extractor. The CO_2 stream then passes to the first separation vessel where the conditions P_1, T_1 are set to precipitate the first fraction of least soluble components. The output stream from the first separator is then passed to a second vessel at lower pressure P_2, where the second fraction precipitates. The fractionation thereby takes place down a 'cascade' of separation vessels set at progressively decreasing pressures. Because separation is much more rapid than extraction the process is not rate-limited. Care must, however, be taken to avoid carrying-over of precipitated material between separation vessels.

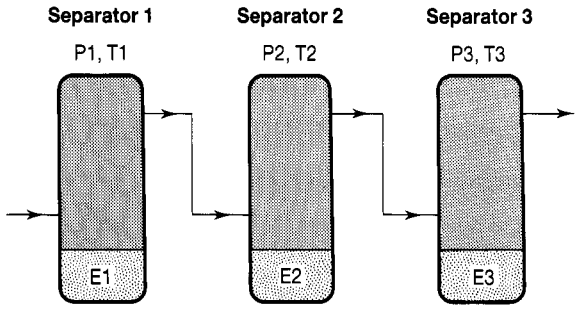


Fig. 2.18. Separation stage configuration for NCF fractionation using a 'cascade' of separation vessels (E = extract).

Zosel's 'hot finger' fractionation column

The anomalous effect of temperature at low pressures in SCFs has been introduced previously (Section 2.3.1) in which increasing the temperature results in a drop in solubility. If the temperature rise is sufficiently high, this behaviour can also be extended

to fluids at higher pressures. Zosel (1978) has exploited this effect in an elegantly simple fractionation apparatus (Fig. 2.19) in which fractionation and extraction take place in a single high-pressure vessel.

The mixture to be fractionated is placed in the bottom of the vessel and a 'hot finger' probe at the top serves to establish a temperature gradient throughout the fluid in the column. Ignoring the small pressure gradient required to maintain flow, the vessel can be considered to be at constant pressure. The density of the fluid and solubility of components are therefore uniquely determined by temperature. Solvation in the high-temperature (low-density) region in the vicinity of the probe will be at a minimum and only the most soluble components will remain in solution and be able to pass through to the separation vessel. Less soluble components will precipitate and remain under reflux in the lower portion of the column. Fractions can be removed from the column in order of decreasing solubility by progressively lowering the temperature of the probe and/or increasing the pressure.

The potential of the apparatus has been demonstrated in the fractionation of fish oils into over 50 discrete fractions.

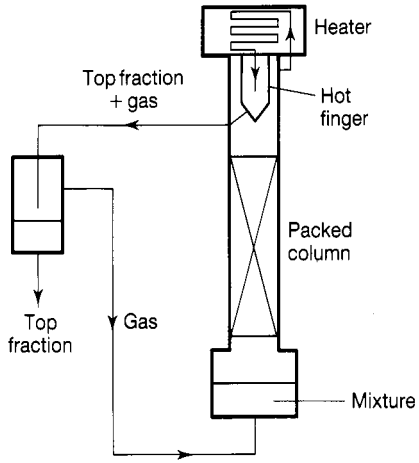


Fig. 2.19. An NCF fractionation column using a 'hot finger' to control selectivity (Zosel, 1978).

2.6 APPLICATIONS

The last decade has seen a rapid growth in research activity in the area of SCF extraction. Although many glowing reviews and hundreds of publications have appeared covering a wide variety of applications, there are surprisingly few processes and plant operating on a commercial scale. It is possible that many of the early publications overstressed the potential advantages of SCFs without addressing the limitations. It is certainly the case that many reports of applications do not differentiate between the extraction/identification of trace amounts of components and realistic quantities on which a process could be

based. Without the necessary data it is often difficult or impossible to quantify the efficiency of a reported SCF extraction process in terms of mass transfer and throughput of CO_2. Against this background it is not surprising that many popular misconceptions abound regarding SCFs.

SCF extraction is an expensive process and, although offering many advantages (Table 2.3), should not be used simply because it is a 'novel' technique. Unless use is being made of its unique features there is no rationale for its implementation if a cheaper separation process can meet the requirements of the separation as effectively. The one clear advantage that CO_2 does offer for food applications is its lack of toxicity. In the current climate of growing consumer concern regarding food safety this feature will undoubtedly promote its use in the food industry.

The following review of applications includes examples of processes currently operating on a commercial scale and applications that could soon be implemented. Examples of applications showing novel features that serve to illustrate the potential of SCF extraction are also included.

Table 2.3. Criteria determining implementation of SCF CO_2 extraction

Criteria	Advantages	Disadvantages
Economic	Can save energy Cheap Stable market	High capital cost
Safety	Non-toxic* Non-flammable	High pressure
Physical properties	Enhanced control through T and P (fractionation*) High vapour pressure enables separation at low T* Low viscosity can provide enhanced mass transfer	Low solubilities
Chemical properties	Non-oxidative Environment*	pH effects and hydrate formation when water present

* = implication for improved quality.

2.6.1 Decaffeination of coffee and tea

Over the past decade there has been a growing consumer aversion to the levels of stimulants in beverages and there is now a large market in decaffeinated products. Although there are some notable differences, the conventional solvent and NCF extraction processes for coffee and tea share many common features.

In the production of coffee 'green' beans are roasted to generate the coffee oils that later impart flavour to the infusion. To avoid co-extraction of flavour components in the decaffeination process green beans are therefore generally extracted. Moist beans are used since it is found that dry beans do not allow effective extraction. The crucial role of water in the process has not been unambiguously established but is thought to 'free' the caffeine from adsorption on the surface, reduce complexation with other molecules and reduce the tortuosity factor by swelling the cellular structure. In the conventional decaffeination process, organic solvents such as methylene chloride or ethyl acetate are employed to reduce the level of caffeine from approximately 1% w/w to 0.06%. Heightened awareness of the potentially harmful effects of residual levels of these solvents has provided some impetus to examine alternative safer solvents such as NCF CO_2. Extensive kinetic studies of the infusion of coffee beans into water (Spiro *et al.*, 1984) and organic solvents (Bischel, 1979) have been reported.

Judging by the scale of its implementation, decaffeination of coffee is one of the most successful commercial applications of CO_2 extraction with large plants operating in West Germany (Bremerhaven, 27.3 million kg per year) and the USA (Dallas). At first sight CO_2 extraction would not appear to be promising since the solubility of caffeine in NCF CO_2 is relatively low (< 0.2% w/w; Ebelling and Franck, 1984), but this is offset by the high added value of the process. Moreover, CO_2 provides a very selective solvent for decaffeination which does not remove as many of the desirable flavour–precursor components as alternative organic solvents (e.g. ethyl acetate).

Selectivity for caffeine is probably greatest in NCL CO_2, though solubility is low (approx. 0.05%). Increasing pressure and temperature serves to increase the solubility of caffeine but reduces the separation factor. In some processes for production of decaffeinated instant coffee, flavour components that are co-extracted are later separated and added back at the drying stage (Roselius *et al.*, 1974). It is often stated that the water added in decaffeination serves to increase the solubility of caffeine in NCF CO_2. This hypothesis is not borne out by experimental evidence, since increasing the levels of water to saturation does not significantly affect the solubility of caffeine in SCF CO_2 (Moulson, 1988).

A comprehensive review of the patent literature on decaffeination (and other NCF extraction processes) is given in an excellent treatise on NCF extraction by McHugh and Krukonis (1986). Most of the methods employed share common features. In a typical process (Zosel, 1974), wet green beans are contacted with SCF CO_2 in an extraction vessel and the extracted caffeine is removed by partitioning to a coexisting aqueous phase. Other methods of separation of caffeine from the CO_2 stream involve adsorption onto active carbon (Roselius *et al.*, 1979). The latter approach is less desirable since the caffeine, which can be sold as a by-product, is destroyed by burning during the reactivation of the carbon adsorbant. Some patents (Prasad *et al.*, 1981; Margolis and Chiovini, 1981) describe techniques for the extraction of aqueous roasted coffee extracts in a continuous counter-current extraction process. One of the more novel claims in the patent literature involves mixing the beans with active carbon and carrying out the extraction and separation in one vessel (Zosel, 1981). This not only saves on capital costs, but also promotes rapid mass transfer. The activated carbon (containing extracted caffeine) is later removed by sieving.

Decaffeination illustrates well the adverse effects of solute adsorption since it is observed that considerably more CO_2 is required to effect decaffeination than would be expected on solubility grounds alone. This is because caffeine adsorbs onto the beans and prevents equilibrium solubility being reached in the extraction vessel (McHugh and Krukonis, 1986).

Decaffeination of coffee represents one of the most widely patented applications of NCF extraction with innumerable variations and permutations claimed. In contrast the NCF decaffeination of tea has been less well documented (Vitzthum and Hubert, 1979). This may well be due to the more delicate flavour profile of tea, which is more susceptible to damage during the extraction process. The higher levels of caffeine in tea (3% compared to 1% for coffee) may also be a contributing factor.

2.6.2 Seed oil extraction

Extraction and processing of seed oils is a large-scale commercial operation with high throughput. The type of oilseeds processed depends almost entirely on regional agricultural policies; in the USA soya oil is by far the largest commodity, whereas in Canada rapeseed oil is more common. However, seed oils all contain the same basic triglyceride units, though the distribution of individual carboxylic acids in the triglycerides is a unique feature of each oil which imparts individual characteristics. The overall solubility of seed oils in NCF CO_2 does not appear to vary much and basic principles and conditions established for the extraction of one oil translate reasonably well to another.

In the conventional process for oilseed extraction the pretreated seeds are extracted using hexane. This also removes phospholipids (lecithin) which, although beneficial to health, present physical problems when the oil is used for cooking. In the refining process a degumming stage is therefore required to remove the phospholipids from the oil which is then bleached (to reduce colour) and deodorised.

Most of the pioneering work on the SCF CO_2 extraction of seed oils has been carried out in the USA and West Germany. The solubility of soya oil in SCF CO_2 has been measured at high pressure (Friedrich *et al.*, 1982; Stahl *et al.*, 1980, 1983a, 1984) and there has been some success in fitting solubility data using simple EOS models (Klein and Schultz, 1989). The most important feature of Friedrich's measurements was that at high pressures and temperatures (800 bar, 70°C) the oil became completely miscible with CO_2. This suggested the possibility of an efficient high-pressure SCF extraction process (Friedrich and Pryde, 1984). Under these extreme conditions the rate of extraction from soya flake was found to be rapid, and nearly complete extraction could be achieved in 20 min. Moreover, since the solubility falls markedly at lower pressures, most of the oil could be separated from the CO_2 stream without having to undergo complete decompression. Other investigators of the rate of NCF extraction of oilseeds have worked at lower pressures (e.g. King *et al.*, 1987; Brunner, 1984).

Probably the most significant feature of the SCF extraction of oilseeds is that phospholipids are not co-extracted (Friedrich and Pryde, 1984). This eliminates the need for chemical degumming of the oil. If the phospholipids are required as a separate commodity they can be extracted from the seeds in a secondary extraction with a solvent

such as hexane. Oil extracted with SCF CO_2 is also often reported as having a lighter colour.

Although showing many advantages over conventional processing techniques (List and Friedrich, 1985) NCF extraction is not currently economically viable for large-scale oilseed extraction. This is due to the low bulk value of seed oils, high plant cost and inconvenience of batch processing large quantities of solid materials under high-pressure conditions. As a result there has been much interest and speculation concerning the development of systems for continuously feeding solids into high-pressure vessels. Although a successful design was originally implemented for transferring coal slurries at low pressure (Blisset *et al.*, 1979) there appears to have been little success in producing a working system for operating under the higher pressures involved in SCF extraction, though many designs have been proposed (Stahl and Stadler, 1984).

Implementation of SCF extraction of bulk commodity seed oils will probably have to await the development of continuous processing plant or the tightening of legislation governing the use of petrochemical solvents. SCF extraction is, however, appropriate for smaller-scale processes involving high-value oils such as evening primrose (Tolboe *et al.*, 1988) and wheatgerm (Christianson *et al.*, 1984) for which there is a growing market in the health sector.

2.6.3 Purification of lecithin

Lecithin is an important emulsifying agent in the food and pharmaceutical industries. Crude lecithin as produced in the degumming of seed oils is a darkly coloured, highly viscous substance with a 'grease-like' consistency. After extraction with acetone, which removes associated oil and other components, the purified lecithin is obtained as a pale yellow powder with a high phospholipid content (95%). The lecithin residue contains significant levels of residual solvent which are removed at high temperatures at which further degradation of the product can occur.

The large differential in solubility between triglyceride oils and phospholipid suggests the feasibility of using NCF CO_2 to purify lecithin. The main problem here is the highly viscous state of lecithin, which presents enormous difficulties in its transfer into extraction vessels, and achieving good solvent contact therein.

Stahl and Stadler (1984) have proposed a process for the continuous extraction of lecithin which involves high-pressure extrusion of the crude lecithin into an extraction vessel through a small nozzle. Another solution to this problem, incorporating some ingenious features, has been reported by Peter *et al.* (1989). The possibility of using NCFs to reduce the viscosity of liquids has been previously mentioned (Section 2.2.1). However, because CO_2 is not very soluble in lecithin it is not possible to use SCF CO_2 to reduce its viscosity. Instead, NCL propane was employed by Peter to thin the lecithin which was then pumped into an extraction vessel containing SCF CO_2 at 55°C, 80 bar, where most of the lecithin was selectively precipitated. By employing temperatures at which the lecithin can be removed as a liquid the plant could be made to operate continuously and at low pressures. The paper presents a model example of an SCF process development study including phase equilibrium measurements, plant design and cost analysis.

2.6.4 Lowering cholesterol levels in foods

Although the correlation between dietary intake and levels of cholesterol in the blood is not universally accepted, initial suspicions have led to strong consumer aversion to high-cholesterol foods. This has been compounded by advertising campaigns claiming low levels of cholesterol, even in products that would not be expected to contain it.

On solubility grounds there would appear to be a good chance of selectively extracting cholesterol from oils and fats using NCF CO_2. Chrastll (1982) has measured and collated the solubilities of a variety of food components in NCF CO_2 and correlated his data using a simple 'mass action' model. Even from the limited pressure range used in this study it is clear that the solubility of cholesterol is significantly greater than that of triglyceride oils.

Krukonis (1988) has tested the feasibility of removing cholesterol from butter, egg yolk and beef tallow by measuring the partition coefficients of the individual components in SCF CO_2. Selectivity at 60°C and 150 bar was found to be greatest for egg yolk (12.2) and least for butter (3.4). This trend is in accord with the relative solubilities of the component oils in SCF CO_2. In these trials 90% removal of the cholesterol from butter was reported with an overall yield of 70% of cholesterol-reduced product. Distribution coefficients of cholesterol between milk fat and SCF CO_2 reported by Bradley (1989) support the viability of selective separation. Studies on the effect of extraction conditions upon the composition of SCF CO_2 extracted egg yolk powder (Froning *et al.*, 1990) are in broad agreement with available solubility data.

2.6.5 Fractionation of high-value oils and fats

Butterfat

Milk fat contains fatty acids representing a wide range of molecular weight and unsaturation. The acid residues are distributed in a 'random' fashion in the constituent triglyceride oils. Conventional fractionation techniques (e.g. crystallisation – see Chapter 8) are unable to effectively concentrate a short chain acid fraction (C_4–C_{10}) since these acids are combined in the triglyceride structure with higher molecular weight acids (C_{14}, C_{16}, C_{18}) which have a controlling influence on the separation. The fractionation of butterfat is of considerable interest in the dairy industry for manipulation of physical properties (e.g. spreadability, mouthfeel) and functionality in other milk products and foodstuffs. Short-chain fatty acids are also of physiological importance since they are more rapidly assimilated in the body.

Kaufmann *et al.* (1982) have examined the 'time-controlled' fractionation of butterfat in an SCF extraction process at 80°C, 200 bar and the two fractions taken showed clearly different compositions of component oils. The first fraction was found to be 81% enriched in the short-chain (C_4–C_{10}) acids with a simultaneous 44% reduction in the oleic acid content. Accordingly the second fraction showed a higher proportion of longer-chain acids and contained 99% of the C_{24}–C_{44} triglycerides.

More recently Kanare *et al.* (1989) have reported the composition of extracts taken using a 'pressure-controlled' SCF extraction process in which the pressure was progressively increased from 100 to 400 bar at 50°C. Analysis revealed a similar pattern to

Kaufmann with the short-chain acids being selectively extracted in the early stages of the extraction. Both cholesterol and lactones were also preferentially removed in the initial extracts.

Fish oils

Certain fish oils, which are rich in the highly unsaturated eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6) acids, are now believed to be of great dietary value. There is therefore considerable interest in the isolation of fractions of fish oils enriched in these component acids. To effect a realistic separation by any technique the acids in the oil must firstly be removed from the triglyceride structure by hydrolysis and esterified, usually as the ethyl ester. The conventional fractionation process then involves distillation techniques which are limited in terms of both thermal degradation of the oils and the separation factors obtainable.

One of the first reports of the application of SCF extraction in the fractionation of fish oils was by Eisenbach (1984) who demonstrated enrichment of the component acids of cod liver oil by carbon number. Further separation of acids differing only by number and position of double bonds could not be achieved. However, the technique was successful in producing a C_{20} fraction containing about five times the concentration of EPA of the starting material.

Nilsson *et al.* (1988) solved the problem of limited separation of EPA in a combined SCF extraction/urea crystallisation process in which less saturated acids (e.g. C20:0, C20:1) can be selectively removed. In this combined process, fractions containing 96% pure EPA were obtained from menhaden (large herrings) oil. In the course of this work distribution coefficients of more than twenty individual acid components were determined in SCF CO_2. The feasibility of this fractionation process has recently been established in a series of pilot scale tests by Krukonis (1988).

2.6.6 Extraction of flavours and fragrances

Flavours and fragrances are conventionally isolated from botanical sources either as an absolute, using solvent extraction, or by steam distillation. The main drawbacks to these methods are thermal degradation (e.g. of sesquiterpenes), loss of volatile 'top notes' and indiscriminate separation of high molecular weight components. It was realised at an early stage in the development of NCF extraction that these problems could be largely overcome by using CO_2.

The main advantages in using NCF CO_2 for the isolation of flavours and fragrances are:

(1) The extraction and separation can be carried out at low temperature in an inert environment, thereby avoiding thermal damage and chemical degradation.
(2) The extract has improved solubility in formulations since less terpenes are extracted.
(3) The high vapour pressure of CO_2 enables it to be removed without losses in the highly volatile 'top notes'.
(4) Undesirable components such as proteins, waxes, sugars, chlorophyll are not extracted.

Most of the early work on the use of liquid CO_2 for flavour extraction was carried out in the Soviet Union in the 1960s and reported in Russian. Since then the variety of flavours and fragrances examined has grown enormously and now represents probably the largest class of researched applications for CO_2 extraction in the food industry. Of all examples, that of hop oil extraction deserves special mention since it is one of the few applications regularly operating on a large commercial scale. In hop oils it is the soft resins consisting of alpha acids (humulones) and beta acids (lupulones) that are the important components for flavour production. In the brewing process alpha acids are isomerised to give beer its characteristic taste. Conventional hop extraction processes use either hexane or methylene chloride and, although residual levels of these solvents are within allowed limits, CO_2 offers many of the above advantages ((1)–(4)) and, being non-toxic, has a far higher level of acceptability.

A process for hop extraction using SCF CO_2 has been patented by Vitzthum *et al.* (1972) and a complementary process using NCL CO_2 was later developed by Laws *et al.* (1980). The latter process uses NCL CO_2 at 7–10°C, where it is claimed that a solubility maximum exists. The hop extract is rich in the desirable acids and essential oils and contains no 'hard' resins. The process was implemented on a commercial scale at Reigate (UK). Similar plants are operating in West Germany at supercritical conditions (Münchsmünster). Although the higher temperature of the SCF process facilitates a more rapid extraction, some selectivity is lost and more chlorophyll is also co-extracted.

Hop extraction is a highly seasonal process and can be carried out only over a limited period. Most commercial hop extraction plants are therefore also used for other applications out of the hop season, e.g. in the extraction of flavours, herbs and spices, and are also often offered for hire on a 'contract' basis.

Judging by the number of publications appearing over the last decade there must be few botanical plants providing flavour and fragrance extracts that have not been subjected to CO_2 extraction. Most tests show an improved quality extract resembling more closely the botanical source for which quantitative evidence is often presented in the form of chromatograms. However, specific information regarding efficiency and mass transfer is often lacking. As in the case of hop extraction liquid CO_2 is found to give a higher quality extract, but this is probably offset by a less efficient extraction. A selection of relevant literature is given in Table 2.4. NCF extraction has also been used to extract flavours and aromas from a variety of other sources including fruit juices (Schultz and Randall, 1970), wine (Jolly, 1981), tea (Vitzthum *et al.*, 1975a), cocoa (Vitzthum *et al.*, 1975b) and tobacco (Luganskaya *et al.*, 1967).

Inexperience of high-pressure technology, lack of design data and high capital costs have all contributed to limit the application of SCF extraction in the food industry. However, selected applications making use of the unique properties of SCFs have been, and are being, profitably exploited. It is noteworthy that the products of such processes, although sometimes more expensive, have found a place in the market by virtue of their improved quality. This reflects the current trends of the consumer towards purer and more natural processed foods. It seems likely that these consumer demands, combined with increasing legislative restrictions, will dictate greater implementation of SCF extraction technology in the future.

Table 2.4. Examples of SCF extraction processes

Plant	Reference
Almonds	Calame and Steiner (1982)
Anise	Stahl and Gerard (1982)
Basil	Pekhov *et al.* (1975)
Bergamot*	
Black pepper*	Moyler (1986), Sankar and Manohar (1988), Calame and Steiner (1982), Vitzthum and Hubert (1971)
Camomile*	Stahl and Schilz (1976), Weust *et al.* (1981)
Caraway	Stahl and Hubert (1976), Stahl and Gerard (1982)
Cardamon*	Meerov *et al.* (1971)
Cinnamon	Stahl and Gerard (1982), Tateo and Gerard (1989)
Clove*	Stahl and Chizzini (1982), Moyler (1986), Gopalakrishnan *et al.* (1990)
Coriander*	Volodicheva and Lybarskii (1974)
Cumin	Gangadhara and Mukhopadhyay (1988)
Fennel	Volodicheva and Lybarskii (1974)
Ginger*	Stahl and Schilz (1976), Pellerin (1988), Sankar and Manohar (1988), Moyler (1986), Chen *et al.* (1986)
Hop*	Moyler (1986)
Horseradish	Stahl *et al.* (1983a or b)
Juniper*	
Lemon*	Calame and Steiner (1982), Temelli *et al.* (1988)
Lime*	
Lovage*	
Mace*	
Marjoram*	Bouclier and Koller (1986)
Nutmeg*	Hubert and Vitzthum (1978), Moyler (1986)
Orange*	
Paprika*	Coenen and Hagen (1983)
Parsley*	
Patchouli*	
Peppermint	Shaftan *et al.* (1973)
Pimento*	Moyler (1986)
Red pepper	Hubert and Vitzthum (1978)
Rosemary*	Pellerin (1988), Tateo and Fellin (1988)
Sage*	Stahl and Shilz (1976), Pellerin (1988)
Sandalwood	Sankar and Manohar (1988)
Thyme*	Bestmann *et al.* (1985)
Vanilla*	Shuetz (1981), Vitzthum and Hubert (1971)

* Commercially available extracts.

Symbols used in text

a = constant in attractive term of EOS
b = constant in repulsive term of EOS
C = concentration
D = diffusion coefficient
D^{eff} = effective diffusion coefficient
f = fugacity
k = binary interaction parameter
K = equilibrium constant
L = half thickness of slab
M_t = mass of solute extracted at time t
M_∞ = mass of solute extracted at infinite time
P_c = critical pressure
R = gas constant
S = solubility
T_c = critical temperature
V = molar volume
x = mole fraction (in liquid or solid phase)
y = mole fraction (in gas or NCF phase)
η = viscosity
τ = tortuosity factor
ϕ = fugacity coefficient
φ = volume fraction of pores

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Chapter 3

Pressure-activated membrane processes

M. J. LEWIS, Department of Food Science and Technology, The University of Reading, Reading RG6 6AP

3.1 INTRODUCTION

Over the last 30 years, a number of membrane processes have evolved, which make use of a pressure driving force and a semi-permeable membrane in order to effect a separation of components in a solution or colloidal dispersion. The separation is based mainly on molecular size, but to a lesser extent on shape and charge. The three main processes are reverse osmosis (hyperfiltration), ultrafiltration and microfiltration. The dimensions of the components involved in these separations are given in Fig. 3.1, and are typically in the range of less than 1 nm to over 1000 nm. A brief summary of the main differences between them, in terms of the components which are rejected by the membranes, is also illustrated. More recently the process term 'nanofiltration' has been introduced, which is somewhere between reverse osmosis (RO) and ultrafiltration, bringing about a separation of low molecular weight components such as monovalent ions and salts from organic

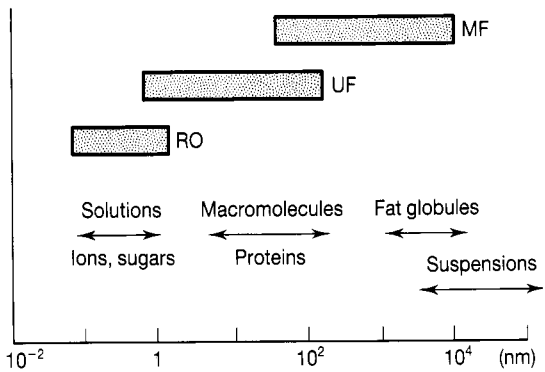


Fig. 3.1. Size ranges for different membrane processes.

compounds such as sugars. These pressure-activated processes can also be regarded as a continuous spectrum of processes, with no obvious distinct boundaries between them. However, it should be noted that the sizes of the components being separated range over several orders of magnitude, so it is highly likely that the separation mechanisms and hence the operating strategies may change as we move through the spectrum.

3.2 TERMINOLOGY

The feed material is applied to one side of a membrane. The feed is usually a low-viscosity fluid, which may sometimes contain suspended matter and which is subjected to a pressure. In most cases the feed flows in a direction parallel to the membrane surface and the term cross-flow filtration is used to describe such applications. Dead-end systems are used, but mainly for laboratory scale separations. The stream which passes through the membrane under the influence of this pressure is termed the permeate (filtrate). After removal of the required amount of permeate, the remaining material is termed the concentrate or retentate. The extent of the concentration is characterised by the concentration factor (f), which is the ratio of the feed volume to the final concentrate volume (see equation (3.5)).

The process can be illustrated simply in Fig. 3.2(a). From a single membrane processing stage, two fractions are produced, named the concentrate and permeate. The required extent of concentration may not be achieved in one stage, so the concentrate may be returned to the same module for further concentration or taken to other modules in a cascade, or multistage process. The permeate may also be further treated in a separate process.

In terms of size considerations alone, one extreme is a membrane with very small pore diameters (tight pores). In this case the permeate will be pure water because even small molecular weight solutes will be rejected by the membrane; high-pressure driving forces are required to overcome frictional resistance and osmotic pressure gradients. If the permeate is predominantly water, then the process is known as reverse osmosis or hyperfiltration; it is similar in its effects to evaporation or freeze-concentration. A concentrate will be produced, in which there is virtually no alteration in the proportion of the

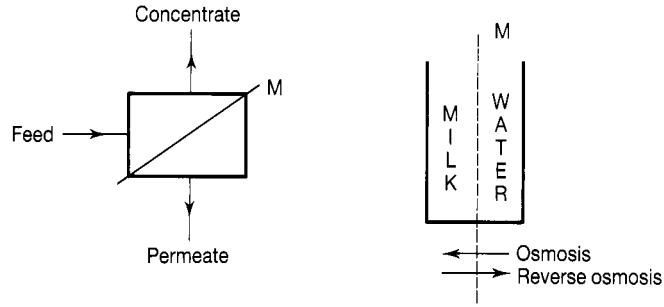


Fig. 3.2. Separation of feed into a concentrate and permeate stream.

solid constituents. In some applications it is the permeate which is the required material; for example the production of 'drinking water' from sea-water or 'pure water' from brackish water. The best processes are those where both the concentrate and the permeate are fully utilised.

There have been several comparisons made between evaporation and reverse osmosis, in terms of capital costs, energy costs and product quality (Renner, 1991). In general terms RO is less energy intensive and can improve product quality. Some limitations are the high capital costs, membrane replacement costs and extent of concentration, which is not as high as that obtainable by evaporation.

If a fluid, for example milk, is separated from water by a semi-permeable membrane (see Fig. 3.2(b)), there will be a flow of water from the water to the milk, in order to equalise the chemical potential of the two fluids; this is termed osmosis. This flow of water can be stopped by applying a pressure to the milk. This pressure that stops the flow is termed the osmotic pressure. If a pressure greater than the osmotic pressure is applied, the water will flow from the milk to the water, thereby reversing the natural process of osmosis and achieving a concentration of the milk. Therefore in reverse osmosis, the pressure applied needs to be in excess of the osmotic pressure. Osmotic pressure (π) is a colligative property, the pressure being dependent upon the number of particles and their molecular weight. In classical terms it is determined from the Gibb's free energy equation:

$$\pi = \frac{RT}{V_m} \ln \gamma X \tag{3.1}$$

where R = gas constant, T = absolute temperature, γ= activity coefficient, X = mole fraction, and V_m = partial molar volume.

For dilute solutions of non-ionisable materials, the Van't Hoff equation can be used

$$\pi = RT(c/M) \tag{3.2}$$

where c = concentration (kg m^{-3}) and M = molecular weight.

For ionisable salts this becomes

$$\pi = iRT(c/M) \tag{3.3}$$

where i = the degree of ionisation, e.g. for NaCl, $i = 2$; for $FeCl_2$, $i = 3$.

This equation predicts a linear increase in osmotic pressure with concentration. However, this relationship breaks down, even at relatively low concentrations, with the relationship between osmotic pressure and concentration becoming non-linear. For example, the osmotic pressure of a 25% serum albumin solution was 300 τ, which is about six times higher than predicted from the Van't Hoff equation. It is also affected by pH.

This non-linear relationship can be represented by Virial type equations:

$$\pi = Ac + Bc^2 + Dc^3 \tag{3.4}$$

where c = concentration and A, B and D are constants. The constants are presented for dextran and whey by Cheryan (1986).

Osmotic pressures are highest for low molecular weight solutes, so the highest osmotic pressures arise for salt and sugar solutions. Concentration of such solutions results in a large increase in their osmotic pressure. On the other hand, proteins and other macromolecules do not produce high osmotic pressures. There will only be small increases during their concentration as well as small differences in osmotic pressure between the feed and permeate in ultrafiltration. Values for osmotic pressures are not easy to find in the literature and a selection of values is given in Table 3.1. A further complication with foods and other biological systems is their complexity, with not just one but many components. *In reverse osmosis the applied pressure must exceed the osmotic pressure, and the driving-force term in reverse osmosis is normally the difference between the applied pressure and the osmotic pressure.* It could be that osmotic pressure is one of the factors that limits the extent of concentration. One suggested experimental method for measuring osmotic pressure is to determine the pressure that would give zero flux, by extrapolation. In ultrafiltration and microfiltration, there is little osmotic pressure difference over the membrane as the low molecular weight components are almost freely permeating (see equation (3.8)).

Table 3.1. Osmotic pressures of some solutions

Solution		Osmotic pressure (bar)
Sugar beet	20° Brix	34.1
Tomato paste	33° Brix	69.0
Apple juice	15° Brix	20.4
Citrus juice	10° Brix	14.8
	34° Brix	69.0
Sucrose	44° Brix	69.0
Coffee extract	28% TS	34.0
Sea-water	3.5% salt	23.2
	15.0% salt	138.0
Milk		6.9
Whey		6.9
Lactose	1% w/v	3.7

Compiled from data in Cheryan (1986) and Lewis (1982).
Some equations for osmotic pressure are given by Cheryan (1986).

As the membrane pore size increases, the membrane becomes permeable to low molecular weight solutes in the feed; even the transport mechanisms are likely to change. Lower pressure driving forces are required as osmotic pressure differences between the feed and permeate are reduced. However, molecules of a larger molecular weight are still rejected by the membrane. Therefore some separation of the solids present in the feed takes place; the permeate contains low molecular weight components at approximately the same concentration as they are in the feed, and the concentrate contains large

molecular weight components at an increased concentration, compared to the feed. Note that some of the low molecular weight components will be retained in the concentrate. It is this fractionation and concentration process that makes the ultrafiltration process more interesting than reverse osmosis, although, as mentioned earlier, there is no sharp demarcation between the processes. More porous membranes still allow not only sugars and salts, but also macromolecules, to pass through, but retain particular matter and fat globules, i.e. greater than 100 nm (see Fig. 3.1); this is termed microfiltration. Because of their increased potential for separating components in mixed feeds, ultrafiltration and microfiltration are covered in more detail in Chapters 4 and 5. However, much of the discussion, particularly that on membrane performance and rejection, will also be pertinent to all three pressure-activated processes. Major points of difference are discussed later in this chapter.

3.3 CONCENTRATION FACTOR AND REJECTION

Two important processing parameters for all pressure activated processes are the concentration factor (f) and the membrane rejection characteristics. The concentration factor is defined as follows:

$$\textit{Concentration factor} \quad (f) = V_F / V_c \tag{3.5}$$

where V_F = feed volume and V_c = final concentrate volume.

The term volume reduction factor (VRF) is sometimes used:

$$\text{VRF} = 100\,(V_F - V_c)/V_F = 100(1 - 1/f) \tag{3.6}$$

Thus a process with a concentration factor of 10 would have a volume reduction factor of 90%.

The permeate volume (V_p) equals the feed volume minus the concentrate volume (assuming no losses)

$$V_p = V_f - V_c \tag{3.7}$$

As soon as the concentration factor exceeds 1, the volume of permeate will exceed that of the concentrate. Concentration factors may range from as low as 1.5 for some viscous materials, to up to 50 for dilute protein solutions, e.g. chhana whey (Jindal and Grandison, 1992). Generally higher concentration factors are used for ultrafiltration than for reverse osmosis, e.g. up to 25–30 for UF of cheese-whey, compared to 5 for RO of cheese-whey.

A mass balance for the process can be applied and is useful for estimating the distribution of components between the permeate and concentrate, or for estimating the losses that are incurred in practical situations.

The *rejection* or *retention factor* (R) of any component is defined as

$$R = c_F - c_p / c_F \tag{3.8}$$

where c_F is the concentration of component in the feed and c_p is the concentration in the permeate.

It can be determined experimentally for each and every component in the feed, by sampling the feed and permeate at the same time and analysing the component in question. It is a very important property of a membrane, as it will influence the extent (quality) of the separation that can be achieved.

Rejection values normally range between 0 and 1; sometimes they are expressed as percentages (0–100%).

when $c_p = 0$; $R = 1$; all the component is retained in the feed

when $c_p = c_F$ $R = 0$; the component is freely permeating.

An ideal RO membrane would give a rejection value for all components of 1, whilst an ideal UF membrane, being used to concentrate a high molecular weight component or remove a low molecular weight component would give respective rejection values of 1 and 0. If the concentration factor and rejection value are known, the yield of any component, which is defined as the fraction of that component present in the feed, which is recovered in the concentrate, can be estimated. Obviously for reverse osmosis, the yield for an ideal membrane is 1.0. Rejection data for membranes and their effects on yield and separation performance will be discussed in greater detail in Chapter 4.

3.4 MEMBRANE CHARACTERISTICS

The membrane itself is crucial to the process. The first commercial membranes were made of cellulose acetate and these are termed first-generation membranes. For food-processing applications, they had some limitations, with temperatures below 30°C and a pH range of 3–6. These were followed in the mid-1970s by other polymeric membranes (second-generation membranes), with polyamides and, in particular, polysulphones being widely used for foods. The resulting improvements in cleaning and hygiene are covered in Section 3.8. It is estimated that over 150 organic polymers have now been investigated for membrane applications. Inorganic membranes based on sintered and ceramic materials are also now available. The physical structure of these membranes is complex, and as most of them are used for microfiltration, their structure is described in more detail in Chapter 5.

The main terms used to describe membranes are microporous or asymmetric. Microporous membranes have a uniform porous structure throughout, although the pore size may not be uniform across the thickness of the membrane. They are usually characterised by a nominal pore size and no particle larger than this will pass through the membrane. In contrast to this, most membranes used for ultrafiltration are of asymmetric type, having a dense active layer or skin of 0.5–1 μm in thickness, and a further support layer which is much more porous and of greater thickness (Fig. 3.3). Overall the porosity of these membranes is high, although the surface porosity may be low, with quoted values in the range 0.3–15% (Fane and Fell, 1987). Often the porous path may be quite tortuous, the distance covered by the solvent or solute being much greater than the thickness of the membrane; the term tortuosity has been used as a measure of this property. The pores are not of a uniform size, as can be seen when viewed under the electron microscope, and are best characterised by a pore size distribution. This

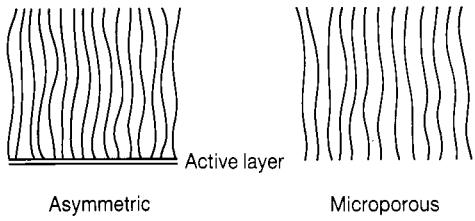


Fig. 3.3. Closer examination of the membrane structure.

distribution can be measured by electron microscope techniques, or by combined bubble size and solvent permeability methods (Munari *et al.*, 1985). It is claimed that this method is capable of measuring the pore-size distribution in the thin skin. Another technique mentioned by Fane and Fell is the capillary condensation/permeability method. Pore sizes may range from 1 to 100 nm. This distribution of pore size is one of the main factors preventing a sharp separation of components of almost similar size, e.g. mono- and di-saccharides (see Rejection). An indirect measurement of pore size can be made by measuring the permeability of solutes, such as dextrans with a range of molecular weights.

It can be seen that in physical terms alone, there are a number of membrane structures available. The membrane also has a chemical nature, and many materials have been evaluated. It may be hydrophilic or hydrophobic in nature. Fane and Fell (1987) stated that the hydrophobic nature can be characterised by measuring its contact angle (θ). The higher the contact angle the more hydrophobic is the surface. Polysulphones are generally much more hydrophobic than cellulosic membranes. There was shown to be a good correlation between flux decline and hydrophobicity, with the least hydrophobic membrane showing the least flux loss over a period of 150 min. The surface may also be charged. All these factors will give rise to interactions between the membrane and the components in the feed and influence the components passing through the membrane, as well as the fouling of the membrane.

The physical chemistry of membranes has been described in more detail by Cheryan (1986), Gutman (1987) and Tsujita (1992).

3.5 PERMEATE RATE

Two other important processing parameters are the flux or permeate rate and the power consumption.

The flux is usually expressed in terms of volume per unit time per unit area ($l\ m^{-2}\ h^{-1}$). Expressed in this way it permits a ready comparison of different membrane configurations of different surface areas. It can also be expressed as a permeate velocity. If energy is to be taken into account, it may be relevant to measure and maximise the flux to energy consumption ratio. Flux values may range from higher than $500\ l\ m^{-2}\ h^{-1}$ to less than $5\ l\ m^{-2}\ h^{-1}$.

(*Note*: Imperial units are still sometimes used, where 1 gal $ft^{-2}\ d^{-1} = 2.036\ l\ m^{-2}\ h^{-1}$.)

Factors affecting the flux rate are the applied pressure: the flow rate and viscosity, both of which affect turbulence, and the processing temperature. Increasing the

temperature and inducing more turbulence increases the flux. However, the flux is only affected by the applied pressure in the pressure-dependent region. These factors are discussed in more detail in Chapter 4.

The power utilisation (W) is related to the pressure (head) developed and the mass flow rate as follows:

$$W = m'hg \tag{3.9}$$

where m' = mass flow rate (kg s^{-1}), h = head developed (m) and g = acceleration due to gravity (9.81 m s^{-2}).

This energy is largely dissipated within the fluid as heat and will result in a temperature rise. Cooling may be necessary if a constant processing temperature is required.

The membrane offers a resistance to the transfer of both solvent (normally water) and solute. The permeate flux is a measure of the flow rate of solvent through the membrane, whereas the rejection describes the amount of solute which passes through (see eq. (3.8)). From a process engineering standpoint, it is highly desirable to be able to predict the flux and rejection from the physical properties of the solution, the membrane characteristics and the hydrodynamics of the flow situation, in order to optimise the performance of the system. Membrane operations have been subject to a number of modelling processes, in order to achieve these objectives. However, before these models are discussed in more detail, it is important to consider the phenomena of concentration polarisation and fouling.

3.6 TRANSPORT PHENOMENA AND CONCENTRATION POLARISATION

A very important consideration for pressure-driven membrane processes is that the separation takes place not in the bulk of solution, but in a very small region close to the membrane, known as the boundary layer, as well as over the membrane itself. This gives rise to the phenomenon of concentration polarisation over the boundary layer. (Note that in streamline flow the whole of the fluid will behave as a boundary layer.) It is manifested by a quick and significant reduction (2–10 fold) in flux when water is replaced by the feed solution, for example in a dynamic start.

Concentration polarisation occurs whenever a component is rejected by the membrane. As a result, there is an increase in the concentration of that component at the membrane surface, together with a concentration gradient over the boundary layer. Eventually a dynamic equilibrium is established, where the convective flow of the component to the membrane surface equals the flow of material away from the surface, either in the permeate or back into the bulk of the solution by diffusion, due to the concentration gradient established. This increase in concentration, especially of large molecular weight components, offers a very significant additional resistance. It may also give rise to the formation of a gelled or fouling layer on the surface of the membrane (see Fig. 3.4). Whether this occurs will depend upon the initial concentration of the component and the physical properties of the solution; it could be very important as it may affect the subsequent separation performance. Concentration polarisation itself is a reversible phenomenon; thus if the solution is then replaced by water, the original water flux should be restored. However, this rarely occurs in practice due to the occurrence of fouling,

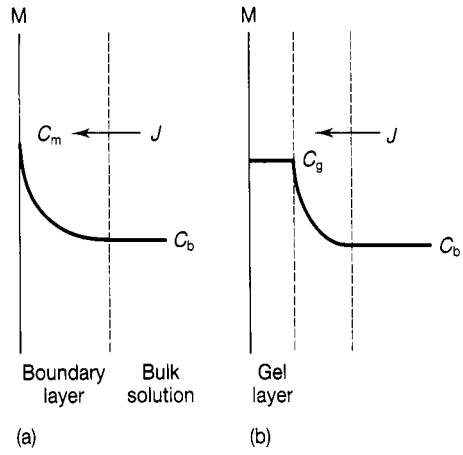


Fig. 3.4. (a) Concentration polarisation in the boundary layer; (b) concentration polarisation with a gel layer.

which is detected by a decline of flux rate at constant composition. Fouling is caused by the deposition of material on the surface of the membrane or within the pores of the membrane. Fouling is irreversible and the flux needs to be restored by cleaning. Therefore, during a concentration process, flux declines due to a combination of these two phenomena.

A number of mechanisms have been proposed to explain the transport of solvent and solute in reverse osmosis. These have been reviewed by Cheryan (1989), and Cheryan and Nichols (1992).

The simplest mechanism to visualise conceptually is a simple sieving mechanism, based on size. However, for reverse osmosis, this does not explain the high rejection of salt and the permeation of water, as the molecules are about the same size. Other physico-chemical factors concerned with the structure of the membrane and interaction of solvent and solutes with the membrane influence the performance. It is believed that for reverse osmosis, the phenomena are more complex than those occurring with ultrafiltration, which is generally regarded as a sieving process. Therefore the models proposed for reverse osmosis and ultrafiltration are different in nature.

One of the most common models used for reverse osmosis is the solute diffusion model, in which the solvent flux (J_w) is influenced in the main by the pressure driving force and the solute flux (J_s) by diffusion. All the resistance to mass transfer takes place in the active layer, which is between 0.5 and 1.0 μm in thickness. In this case the pressure driving force is the difference between the applied pressure and the osmotic pressure. The transport of solvent and solute are not connected. It is assumed that the solute dissolves in the skin instantaneously and then passes through the pores of membrane by diffusion, whereas the flow of solvent (which also dissolves) is influenced by the pressure differential. The relationship between concentration dissolved in the membrane at its surface and that in the solution depends upon the partition coefficient for that component.

Thus the diffusivity and membrane thickness and its partition coefficient all influence the transport of solute. (Convective flow of solute is ignored at high solute rejections.)

According to this model, increasing the pressure will have a preferential effect on solvent flow, thereby reducing solute concentration in the permeate and increasing rejection. It also predicts that increasing the solute concentration in the feed preferentially increases solute transport and increases the concentration in the permeate, thereby decreasing rejection. Increasing the temperature increases both solvent flux and solute flux by about the same amounts, thereby leaving the rejection unchanged. A 1°C change in temperature changes the flux (solute and solvent) by approximately 3%.

Although many of these trends are observed with simple solutions, the theory does not account for all the observed facts for multi-component solutions, whereby the presence of one component increases the permeability of other components. Glover (1985) pointed out that this theory does not describe the behaviour of complex systems precisely, but it gives a background of understanding. In multicomponent systems, differences in the partition coefficients could explain the difference in permeabilities between different components.

A second proposed model is the preferential adsorption, capillary flow model, which predicts that the component concentrated preferentially in the permeate will be that component which is adsorbed most strongly on the membrane surface. The component that is preferentially adsorbed onto the membrane surface provides a thin layer of that component adjacent to the surface. This thin layer then moves through the pores of the membrane by capillary flow, under the influence of a pressure gradient, and in this way is thus preferentially transported through the membrane. For membranes of a hydrophilic nature, the component preferentially absorbed and transported is water. Thus it is postulated that there is a thin film of water adjacent to the membrane surface. This theory also explains the low rejections and sometimes negative rejections for highly polar organic solutes, found with cellulose acetate membranes, due to their preferential adsorption on the surface. Other situations, where rejection of organic solutes decreases with increasing pressure, are explained by their adsorption onto the more hydrophobic regions of the membrane.

A third model is based upon the wetted surface mechanism, whereby water adsorbs onto the surface of the membrane by hydrogen bonding. It is postulated that these clusters of water prevent solute entering the pores and that the water passes through the membrane from one adsorbed site to the next. The energy requirements for water migration are much less than salt migration, thereby promoting separation of the salt and water. All these models are based on knowing the transport mechanisms involved. The physical chemistry of a wide variety of membrane materials, including permeability data, diffusion data and sorption data which are required for the models described earlier, have been reviewed by Tsujita (1992). Also reviewed are transport properties related to non-equilibrium thermodynamics with uncharged and charged membranes.

Other models are based upon irreversible thermodynamics, where the driving force for transport of solvent and solute is expressed in terms of differences in their chemical potential over the membrane. However, the fluxes for solvent and solute are coupled and the flux for each component is influenced by the chemical potential difference for both components. With such models, the exact mechanisms are not known, but the

relationship between the fluxes and driving forces are described by phenomenological coefficients, which are nevertheless connected with the mechanism. Such phenomenological models provide an empirical description of the transport of molecules through membranes, but they do not give an explanation for the separation mechanism. Gekas (1992) discusses these types of model in more detail for membrane processes.

Cheryan (1989) has concluded that none of the proposed models can satisfactorily explain the rejection characteristics of all solvent–solute–membrane reverse osmosis systems.

On the other hand, *ultrafiltration* is regarded as a sieving process and is often modelled as a bundle of capillaries, with flow through the capillaries being described by the Hagen–Poiseuille equation:

$$Q = d^4 \Delta P \ \pi/128\mu L \tag{3.10}$$

where Q = volumetric flow rate, ΔP = pressure drop; μ = dynamic viscosity, L = capillary length and d = capillary diameter. (Note that this equation is often expressed as flux rate/unit area ($d^2 \Delta P/32\mu L$).)

Additional terms may be introduced to account for porosity and tortuosity effects in the membrane.

These equations predict that the flow will be strongly biased toward the largest pores. Such pores may plug quicker because of the higher degree of polarisation encountered due to the higher flux. (See concentration polarisation.) Also, as pore size increases, the flow rate through the membrane will also increase.

The flux for most food solutions is lower than that for water and other pure solvents for a number of reasons. The viscosity of the feed solution may be higher and this further increases throughout the concentration process. Also concentration polarisation and fouling occur. Factors which influence concentration polarisation and fouling are the prevailing flow conditions and the composition of the material being processed. Some of these factors are discussed in more detail in Chapter 4.

3.7 MEMBRANE EQUIPMENT

Membrane suppliers now provide a range of membranes, each with different rejection characteristics; for reverse osmosis salt rejection values are quoted, e.g. from 99% rejection of sodium chloride down to 60% rejection of sucrose; and for ultrafiltration, different molecular weight cut-offs in the range 1000 to 500 000. For example, it is implied that a membrane with a molecular weight cut-off of 20 000 will reject components with a molecular weight greater than that value. Such figures are provided for guidelines only (see Section 4.2.1). Tight ultrafiltration membranes have a molecular weight cut-off value of around 1000–5000, whereas the more 'open' or 'loose' membranes have a value in excess of 100 000. Thus molecular weight cut-off value is related to porosity and rejection characteristics; as membranes become more permeable to solutes, their molecular weight cut-off values increase. However, because there are many other factors that affect rejection, molecular weight cut-off should only be regarded as giving a relative guide to its pore size and true rejection behaviour. Experimental determinations should always be made on the system to be validated, at the operating conditions to be used.

Other desirable features for membranes to ensure commercial success with food components are listed as

- Reproducible pore size from batch to batch, offering uniformity in terms of both their permeate rate and their rejection characteristics.
- High flux rates and sharp rejection characteristics.
- Compatibility with processing, cleaning and sanitising fluids.
- Resistance to fouling.
- An ability to withstand temperatures required for disinfecting and sterilising surfaces, which is an important part of the safety and hygiene considerations. Extra demands placed upon membranes used for food processing include: the ability to withstand hot acid and alkali detergents (low and high pH), temperatures of 90°C for disinfecting or 120°C for sterilising and/or widely used chemical disinfectants, such as sodium hypochlorite, hydrogen peroxide or sodium metabisulphite. The membrane should be designed to allow cleaning both on the feed/concentrate side and the permeate side. See Section 3.8.

Membrane processing operations can range in their scale of operation, from laboratory bench-top units, with samples less than 10 ml, through to large commercial scale operations, processing more than 50 $m^3 h^{-1}$. Furthermore, the process can be performed at ambient temperatures, which allows concentration without any thermal damage to the feed components.

The process can be batch or continuous; the fluid can be static or in motion, either agitated in a stirred cell or moving across the surface of the membrane. The membrane itself can be configured in a variety of forms.

3.7.1 Membrane configuration

The membranes themselves are thin and in most cases require support against the high pressure. The support material itself should be porous. The membrane and its support, together, are normally known as the module.

There are a number of criteria that have to be satisfied in the design of a pressure-activated membrane module. It must provide a large surface area in a compact volume; it must be able to support the membrane and the configuration must allow suitable conditions to be established, with respect to turbulence, high wall shear stresses, pressure losses, volumetric flow rates and energy requirements, thereby minimising concentration polarisation. Hygienic considerations are important; there should be no dead spaces and the module should be capable of being in-place-cleaned on both the concentrate and the permeate side. The membranes should be readily accessible, for both cleaning and replacement. It may also be an advantage to be able to collect permeate from individual membranes in the module to be able to assess the performance of each one individually.

The two major configurations which have withstood the test of time are the *tubular* and the *flat-plate* configurations. The main features of these configurations will be further discussed.

Tubular membranes come in a range of diameters. In general tubes offer no dead spaces, do not block easily and are easy to clean. However, as the tube diameter increases, they occupy a larger space, have a higher hold-up volume and incur higher

pumping costs. The two major types are the *hollow fibre*, with a fibre diameter of 0.001–1.2 mm and the wider tube with diameters up to 25 mm, although about 12 mm is a popular size. Some of the configurations are shown in Fig. 3.5 and 3.6.

For the hollow fibre system, the membrane wall thickness is about 250 μm and the tubes are self-supporting. The number of fibres in a module can be as little as 50 but sometimes greater than 1000. The fibres are attached at each end to a tube sheet, to ensure that the feed is properly distributed to all the tubes. This may give rise to pore plugging at the tube entry point. Prefiltration is recommended to reduce this. The feed is usually pumped through the tubes but it can also be pumped through the shell-side. In general the length of the fibres and modules is not more than 1 m. The geometry of the system ensures that there is a large surface area to volume ratio and that hold-up volumes are low. Pressure drops required are also not excessive because of the short tube lengths. In ultrafiltration applications, the applied pressures are fairly low (usually less than 2 bar). This permits only low inlet pressures, which limits the flow rate that can be achieved as well as restricting its application to low viscosity fluids. However, they are widely used for desalination and in these RO applications are capable of withstanding high pressures. For these applications the outer diameter should be 2–4 times higher than the inner diameter. It is the ratio of the external to internal diameter, rather than the membrane wall thickness, which determines the pressure that can be tolerated (Nishimura and Koyama, 1992).

Hollow fibre systems usually operate in the streamline flow regime. However, the wall shear rates are high. They may not perform well with viscous feeds and those containing particulate matter.

One big advantage is their backflushing capability, which helps improve their cleanability. This is due to the self-supporting nature of the membrane.

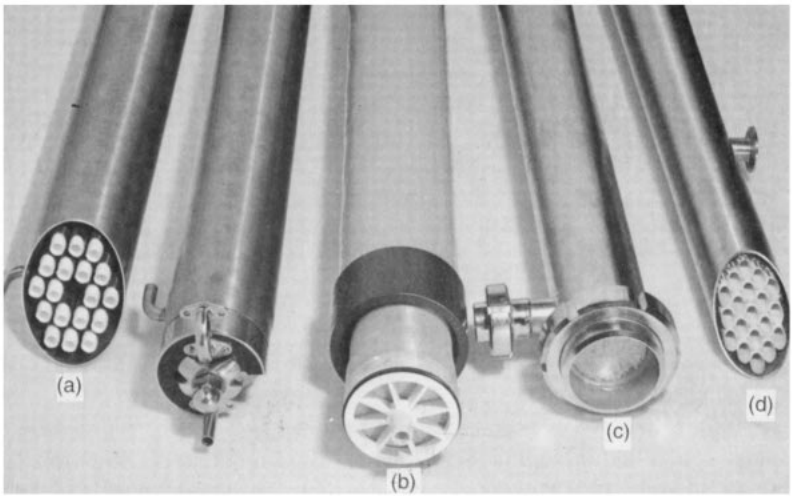


Fig. 3.5. Plate showing different configurations: (a) and (d) tubular; (b) spiral wound; (c) hollow fibre (with courtesy of PCI).

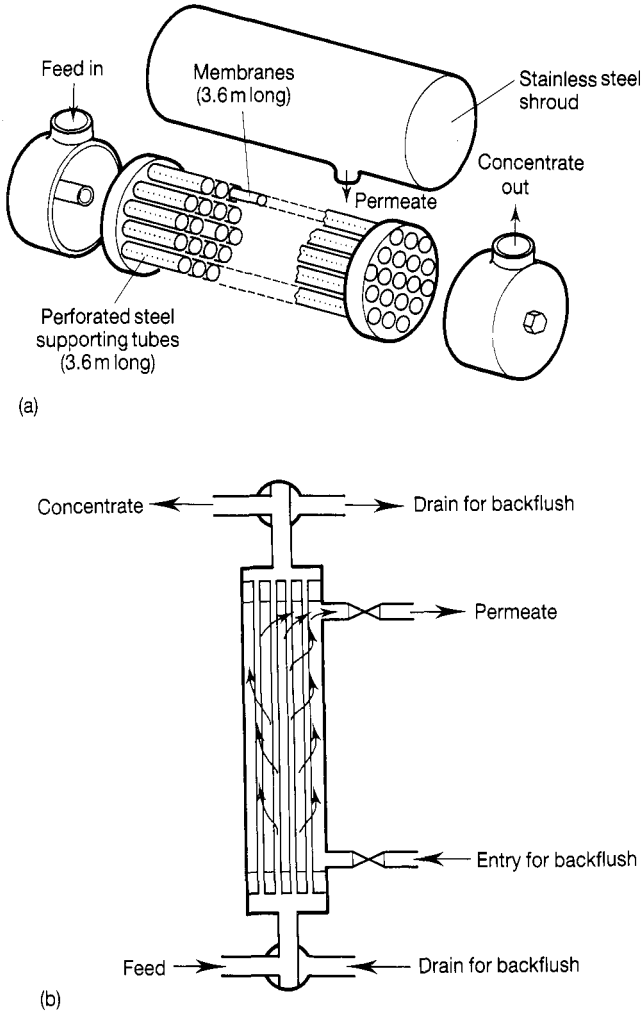


Fig. 3.6. Tubular and hollow fibre configurations: (a) tubular, 12.5 mm diameter, 3.6 m long. Module has 18 tubes, total area 2.5 m^2 (with courtesy of PCI); (b) hollow fibre, length 1.1 m, diameter 7.6 cm, fibres 0.5 mm or 1.0 mm; total area 1.4 or 2.5 m^2. Each module has 1000 or more fibres (with courtesy of Romicon).

Hollow fibre systems tend to be expensive, because if one or several fibres burst, the whole cartridge needs to be replaced.

For *wider tubes*, the feed is normally pumped through the tube, which may be up to 25 mm diameter, although a popular size is about 12 mm diameter. There may be up to 20 tubes in one module; tube lengths may be between 1.2 m and 3.6 m (4 and 12 ft) and tubes within the module may be connected in series or parallel. The membrane is cast or inserted into a porous tube which provides support against the applied pressure. Cheryan

(1986) describes different systems in more detail. Therefore they are capable of handling more viscous fluids and even materials with small suspended particles, up to one-tenth the tube diameter. They normally operate under turbulent flow conditions with flow velocities greater than 2 m s^{-1}. The corresponding flux rates are high, but pumping costs are also high, in order to generate the high volumetric flow rate required at the operating pressure. They are less susceptible to fouling and relatively easy to clean and disinfect by clean-in-place (CIP) methods.

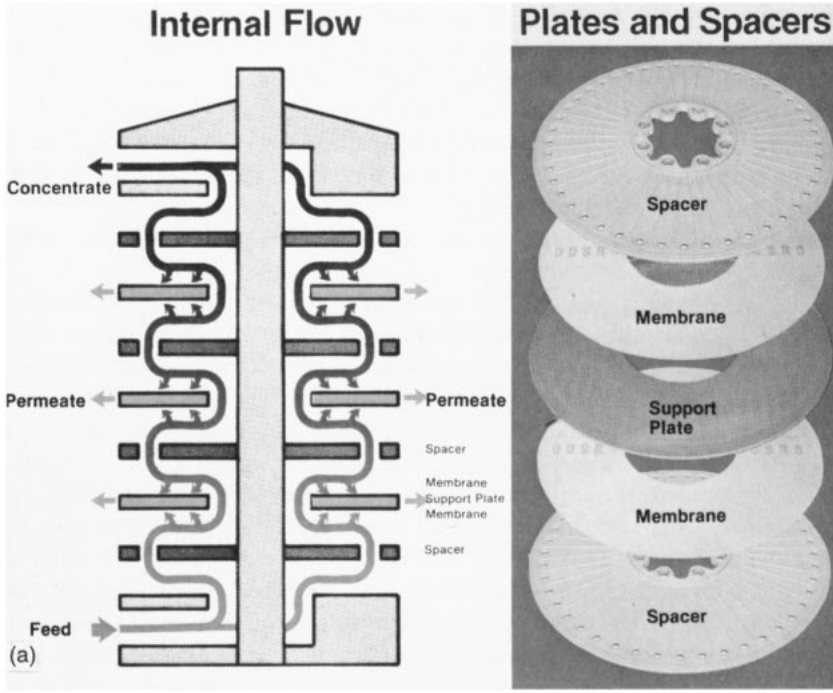
The *flat plate* module can take the form of a plate and frame-type geometry or a spirally wound geometry.

The plate-and-frame system employs membranes stacked together, with appropriate spacers and collection plates for permeate removal, somewhat analogous to plate heat exchangers. The channel height can be between 0.4 and 2.5 mm. Flow may be either streamline or turbulent and the feed may be directed over the plates in a parallel or series configuration. This design permits a large surface area to be incorporated into a compact unit. These systems have been developed for laboratory, pilot plant and large-scale processes, and their performance depends upon the hydrodynamics of the system.

One commonly used system commercially is the DDS plate-and-frame system. Here the channel height is between 0.4 and 1 mm. One membrane is fixed on both sides of a permeate collection plate to form a sandwich. Between each sandwich is a spacer plate (see Fig. 3.7(a)). This system usually operates under streamline flow conditions and the energy required is somewhere between that of a tubular and spirally wound system. Membranes are easily replaced and it is easy to isolate any damaged membrane sandwich. Considerable attention has been devoted to the design of the plate to improve performance. This has been achieved by ensuring a more uniform distribution of fluid over the plate, by increasing the channel width of the longer channels and reducing the ratio of the longest to the shortest channel length. More details are provided by Glover (1985). Also shown in Fig. 3.7(b) is a Pellicon system, used for filtering between 2 and 200 litres.

The *spiral wound system* is now widely used and costs for membranes are quite low. In this case a sandwich is made from two sheet membranes, which enclose a permeate spacer mesh. This is attached at one end to a permeate removal tube and the other three sides of the sandwich are sealed. Next to this is placed a feed spacer mesh and the two together are rolled round the permeate collection tube in the form of a Swiss roll (see Fig. 3.8). The channel height is dictated by the thickness of the feed spacer. Wider channel heights reduce the surface area to volume ratio, but reduce the pressure drop.

The typical dimensions of one spiral membrane unit would be about 12 cm in diameter and about 1 m in length. Up to three units may be placed in one housing, with appropriate spacers to prevent telescoping, which may occur in the direction of flow and could damage the sandwich. Another practical aspect which has received attention is excessive by-passing of feed between the periphery of the membrane sandwich and the housing. Reducing this completely by use of a seal at the inlet improves the performance, but causes a dead space, which may be difficult to clean. Meshes or seals which allow limited by-pass provide a reasonable compromise. This configuration is becoming very popular and relatively cheap. Again the flow may be streamline or turbulent. Pressure drop/flow rate relationships suggest that flow conditions are usually turbulent.



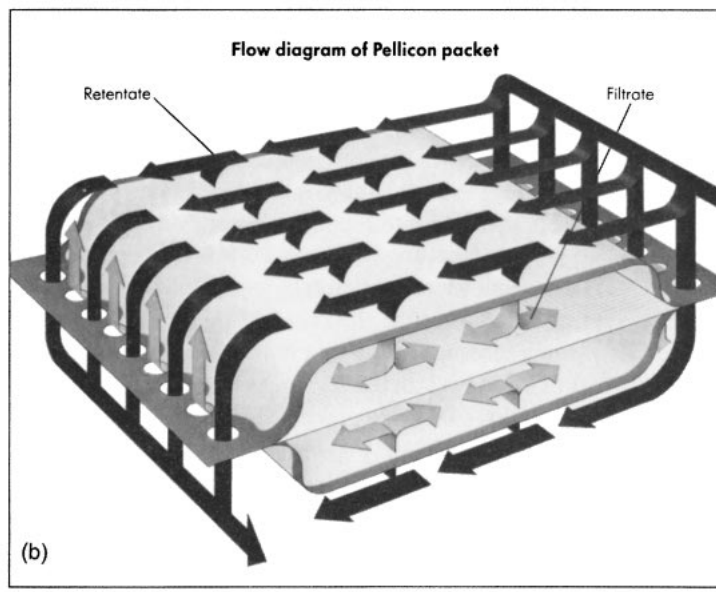


Fig. 3.7. (a) DDS plate arrangement; (b) Pellicon arrangement; with courtesy of DDS and Millipore.

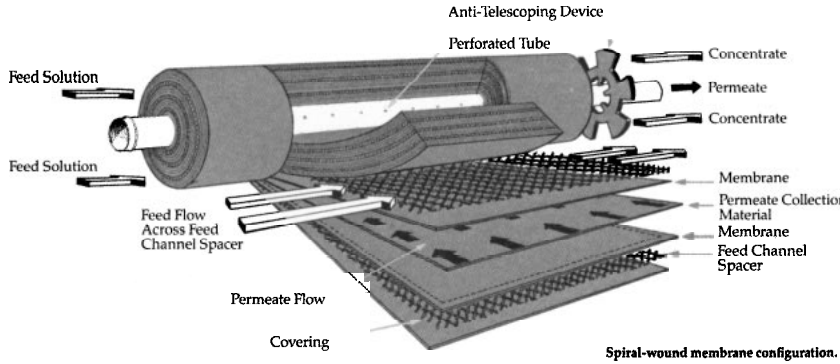


Fig. 3.8. Spiral wound configuration (Courtesy of Abcor).

Both these flat plate arrangements offer a large surface area to volume ratio and low hold-up volumes, as also does the hollow fibre unit. One consequence of this is that a higher degree of concentration usually results after one pass through the module. However, both tubular and flat plate configurations are used in commercial processing operations.

An alternative, much used unit for simple laboratory separations is the stirred cell with agitation facilities. In contrast to systems described earlier, this is a dead-end rather than a flow-through system.

It is beyond the scope of this chapter to make comparisons between the different systems and there have been many articles devoted to comparing their performance. However, an overriding consideration is the constraints imposed by the physical properties of the feed material (viscosity and suspended particles) and the extent of concentration required. Other practical considerations such as high flux rates, long processing runs and ease of cleaning are also important. Additional factors that will influence selection are initial capital cost, membrane life, membrane replacement costs, performance with the test solution in question, reliability, power consumption, local availability, and quality of support and after-service. Each system does and will continue to have its devotees.

As well as the membrane module, there are pumps, pipeline, valves and fittings, gauges, tanks, heat exchangers, instrumentation and control and perhaps in-place cleaning facilities. For small installations, the cost of the membrane modules may only be a relatively small component of the total cost of the finished plant, once these other items have been accounted for. This may also apply to some large installations such as water treatment plants, where other separation processes are numerous and the civil engineering costs may also be high.

Most membrane plants are built on a modular basis, so it is possible to generate data required for scale-up from a single module, under different operating conditions, in order to determine how flux rate is influenced by pressure, temperature, flow rate and total solids. The effects of fouling and any problems with cleaning can also be established. This type of approach will help to establish the best plant layout, as there are a number of

options available. Membrane processes can be operated under batch or continuous conditions.

The simplest system is a batch process. The feed is usually recycled, as sufficient concentration is rarely achieved in one pass. Flux rates are initially high but decrease with time (see Chapter 4). Energy costs are high because the pressure is released each time. Residence times are long. Batch operations are usually restricted to small-scale operations. Batch processing with top-up is used in situations when the entire feed volume will not fit into the feed tank. Batch processing times can be estimated (predicted) if the relationship between flux rate and product concentration is known. In many cases there is a linear relationship between the flux and the log of the concentration. See Chapter 4.

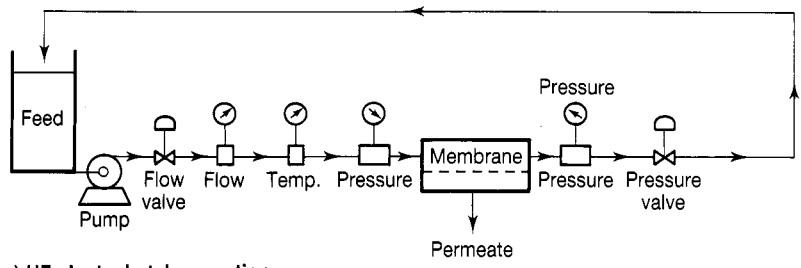
Continuous processes may be single-stage (feed and bleed) or multistage processes, depending upon the processing capacity required. The simplest continuous system is a single-stage process with recycle. Once the retentate has reached its final concentration, a feed and bleed system is operated. Steady state conditions are achieved and product is withdrawn and replaced by fresh feed, at a rate which keeps its composition constant. One drawback is that the concentration in the module is the same as the final product concentration, so the process is operating at its highest total solids content. Therefore, flux rates are low and solids yields may be reduced. The instrumentation and ancillaries for a batch process and a single-stage continuous process are shown in Fig. 3.9.

Some of these problems can be alleviated by multistage processes. In multistage processes, the feed may pass once through each stage (single pass), or be recycled within the stage. Single-pass operations are used in situations where a high degree of concentration is achieved in one pass through the stage. Within each stage, the modules may be arranged in parallel or series. The stages are then arranged in series, with a pump or bleed system between the stages. There may be up to six such stages arranged in series in some larger plants. Figure 3.9(c) illustrates a three-stage process. Each stage contains 24 modules, four banks in parallel, each containing six modules in series.

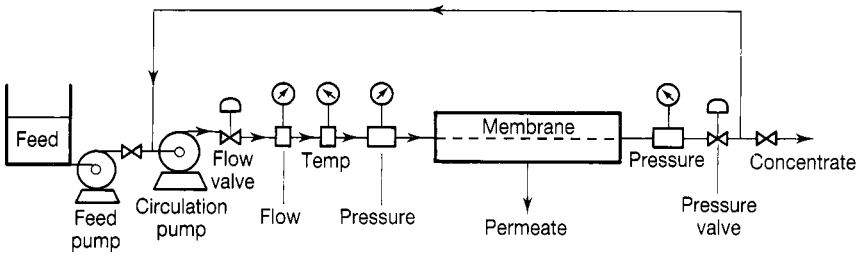
One aspect of continuous processing is that the yield is always lower than that for the equivalent batch process. Also the viscosity increases significantly in the end stages and the volume to be processed decreases. Processing plant with an area greater than 200 m^2 is now commonplace. Figures 3.10 and 3.11 illustrate two commercial installations, based on tubular and plate and frame systems, respectively.

3.8 SAFETY AND HYGIENE CONSIDERATIONS

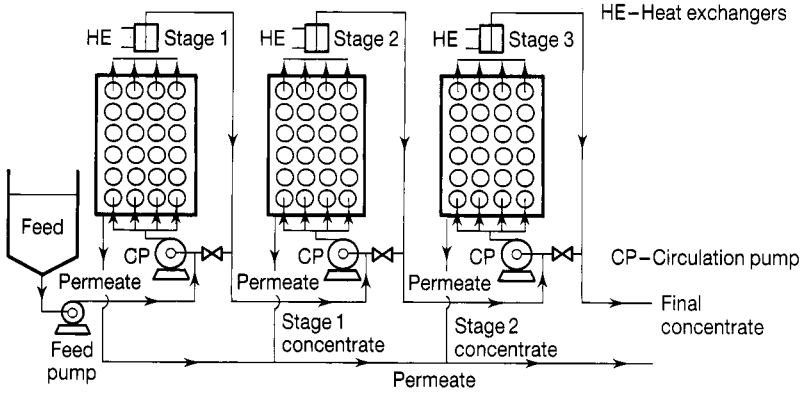
It is important that safety and hygiene are considered at any early stage when developing membrane processes. These revolve round cleaning and disinfecting procedures for the membranes and ancillary equipment, as well as monitoring and controlling the microbial quality of the feed material. For many processes, thermisation or pasteurisation are recommended for feed pretreatment. Microfiltration may also be considered for heat-labile components. From a hygienic viewpoint, the compatibility of the membrane with heat, disinfecting, sterilising and cleaning fluids is critical to the process. There have been considerable advances with this over the last 30 years, since the introduction of *first-generation* membranes based on cellulose acetate. These had limitations; they were not



a) UF plant – batch operation



b) UF plant – continuous, internal recycle or feed and bleed



c) UF plant , commercial scale

Fig. 3.9. Instrumentation for membrane equipment: (a) batch operation; (b) continuous internal recycle or feed and bleed; (c) multistage plant, commercial scale (Courtesy of PCI).

easy to clean and were only recommended up to 30°C. Also they have a poor tolerance to hypochlorite as the chlorine oxidises the cellulose acetate. However, they are still used occasionally because of their high solvent flux and high salt rejection, rarely exceeded by other membranes. Vast improvements were made with the advent of second generation

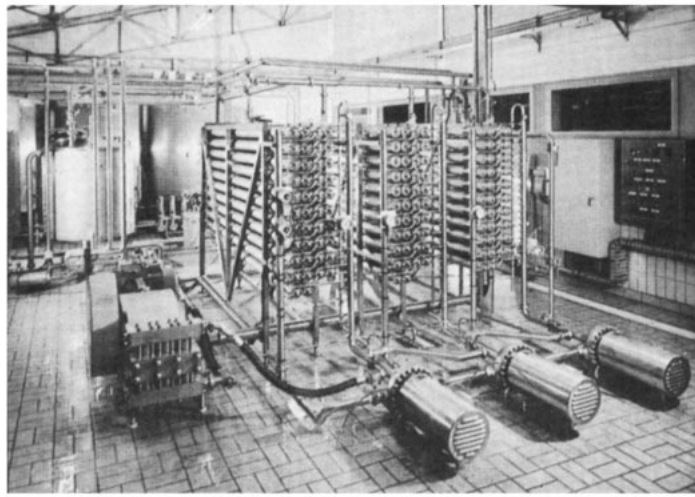


Fig. 3.10. Tubular plant for processing fruit juice (Courtesy of Paterson Candy International).

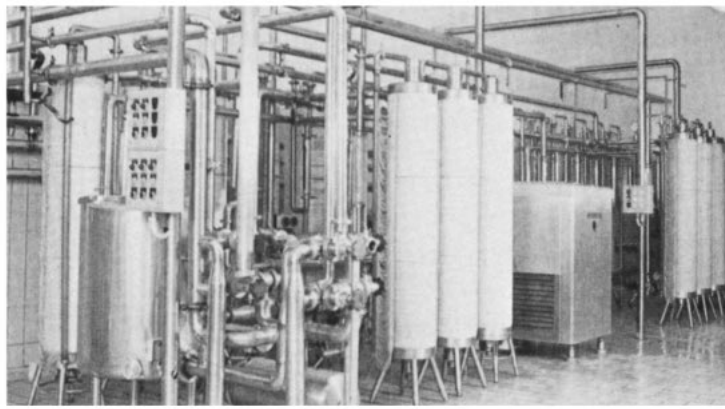


Fig. 3.11. Flat plate plant for processing beer (Courtesy of DDS).

membranes based on polyamides (low tolerance to chlorine) and particularly polysulphones. These are much more tolerant to acids, alkalis and disinfecting temperatures (60–100°C), making it possible to clean and disinfect them effectively, to the standards required in dairy processing. They will also withstand 200 ppm chlorine for short periods and 50 ppm chlorine for long-term storage. The cleaning conditions depend upon the type of produce and the membrane used and need to be established experimentally for each application. In all cases the usual procedure is to flush out all the product with water before initiating cleaning. For fruit juice clarification, a caustic wash at 55°C, followed by a second caustic wash with 200 ppm of hypochlorite, is recommended. Cheryan (1986) reports that hypochlorite helps remove fouling deposits from the pores of

the membrane by causing them to swell. Hypochlorite is also a good cold sterilising agent. *However, as some organic membranes have a low tolerance to chlorine, care should be taken to abide with the cleaning instructions provided by the membrane supplier.* For dairy products, a caustic wash at 50°C to remove fat and protein followed by an acid wash at 50°C to remove minerals is often used. Proteolytic enzymes are useful in cases where heavy protein fouling may be found.

Probably the most effective method of assessing a cleaning regime is to restore the water flux to its original value. However, there is evidence that this does not always ensure that the membrane is totally clean. Successful commercial exploitation depends upon ensuring that the membrane can be properly cleaned, the flux restored. Even under these rigorous conditions, membrane lives of well over one year have been reported.

After cleaning the membrane needs sanitising or disinfecting. Again a combination of heat treatment (up to 100°C), and cold sanitising fluids based on hypochlorite or hydrogen peroxide, are available. One problem with chemical cleaning fluids is that they need to be washed out with water. If the membrane is not to be used immediately, a dilute solution of sodium metabisulphite can be used for storage.

Relatively little has been reported about the *microbiological hazards* associated with membrane processing. Yeasts and moulds will cause problems with acidic products, whereas bacteria will require attention with low-acid products. *All micro-organisms will be rejected by the membrane and will therefore increase in the concentration, by the concentration factor.* There may also be some microbial growth during the process, so the residence time and residence time distribution should be known, as well as the operating temperature. If residence times are long, it may be advisable to operate either below 5°C or above 50°C, to prevent further microbial growth. The feed should be thermised or pasteurised prior to ultrafiltration. Further heat treatment after membrane processing may also be required.

Provided the membrane is not damaged, the permeate should be sterile. However, if not properly cleaned, permeate residues will provide nutrients for microbial growth. Membrane units should be designed to allow cleaning and sterilising on both the feed/concentrate and the permeate sides.

Although little has been written on the subject, the principles involved in *Good Manufacturing Practice* (GMP) (IFST, 1991) and *Hazard Analysis Critical Control Points* (HACCP) (ICMSF, 1988), can be applied. The philosophy is based on improving safety and quality by prior consideration of all sources of microbial hazards, which could be associated with the raw materials and the methods of processing. The process is analysed in detail and a flow chart is produced. This can be used to identify the potential hazards. Once these have been identified, control criteria can be selected for each hazard, with appropriate monitoring systems, to ensure that the process is under control. Finally it is necessary to document all the procedures and verify that the process is under control by monitoring the microbial quality of the final product.

To summarise, the following details should be considered for membrane processes.

- *Raw material quality and treatment*: microbial quality, pasteurisation or other heat treatment, filtration, centrifugation.

- *Processing conditions*: membrane type, operating temperature, pH, residence time and distribution of residence times, all processing parameters should be recorded.
- *Finished product treatment*: further heat treatment, storage conditions.
- *Cleaning*: temperature, time, detergent concentrations; check to ensure restoration of water flux.
- *Disinfecting/sterilising*: times, temperatures, concentrations; check efficacy by taking swabs.

Appropriate control criteria for each of these procedures need to be established, together with monitoring systems for ensuring that the process is kept under control. It is also important to verify this. All these procedures should be documented and the end-product should be monitored to ensure that the product meets with the appropriate microbial standards.

3.9 REVERSE OSMOSIS APPLICATIONS

3.9.1 Introduction

The main applications of reverse osmosis (RO) are for concentrating fluids by removal of water, thereby competing with processes such as vacuum evaporation or freeze-concentration. RO permits the use of lower temperatures even than vacuum evaporation, it avoids a phase change and complete loss of volatiles and it is very competitive from an energy viewpoint.

RO uses much higher pressures than other membrane processes, in the range 20–80 bar, and will incur greater energy costs. Suitable high-pressure pumps will be required, which are normally of the positive displacement type, such as piston pumps. These are expensive and contribute a significant component of the capital costs.

Areas where evaporation is widely used include the dairy, fruit juice and sugar processing industries. Rejection characteristics for different RO membranes are provided in terms of salt rejection; typically from 80 to 99% rejection of sodium chloride; rejections of other solutes may also be cited, for example calcium chloride and glucose.

Products of RO may be subtly different to those produced by evaporation, particularly with respect to low-molecular weight solutes, which might not be completely rejected, and to volatile components, which are not completely lost. (Note that some evaporation plant has aroma recovery facilities.)

Reverse osmosis membranes were made for a long time from cellulose acetate. More recently, thin-film composite membranes, based on combinations of polymers, have been introduced, which allow higher temperatures (up to 80°C) and greater extremes of pH (3–11) to be used, thereby facilitating cleaning and disinfection. Note, however, that those based on polyamides have a very low tolerance to chlorine. However, their performance can often be significantly different. For example, Sheu and Wiley (1983) found that the thin film composite membranes were more efficient in retaining flavours than cellulose acetate, during apple juice concentration. There were also differences in salt rejections and organic molecules and these results together with developments in both cellulose acetate and thin-film composite membranes have been covered by Gutman (1987).

Therefore the main applications of reverse osmosis are for concentrating liquids, recovering solids and treatment of water. Some of these applications will now be reviewed.

3.9.2 Water treatment

Perhaps the most important application is for production of potable water from either brackish water or sea water, by the process of *desalination*. In this case it is the permeate that is the product of interest. Reverse osmosis is used in many areas world-wide, where there are shortages of fresh water. It has been estimated that by 1987, 25% of desalination capacity world-wide was provided by reverse osmosis (Nishamura and Koyama, 1992). In that year, it was estimated that over 3 000 000 m^3 per day of potable water was being produced by reverse osmosis. However, it is still well exceeded by multistage fractional distillation.

Potable water should contain less than 500 ppm of dissolved solids. Brackish water (e.g. borehole or river water) typically contains from 1000 up to about 10 000 ppm of dissolved solids, whereas sea water contains upwards of 35 000 ppm dissolved solids; it can be seen that brackish water treatment requires about 95% reduction of solids whereas sea water requires about 99% reduction of solid matter. Sea water is also a more difficult fluid to process and poses more problems than brackish waters, firstly because of the higher osmotic pressure of 40 bar as opposed to about 5 bar for brackish waters and secondly its more serious long-term effects on the membrane performance. Most of the early reverse osmosis units in the late 1960s and early 1970s processed brackish waters, because there were not membranes available to tolerate the high osmotic pressures involved with sea water (40 bar).

Another interesting feature of both types of plant is that the feed is pretreated by sedimentation, pH adjustment, sand filtration and even microfiltration, in order to reduce fouling to a minimum and ensure that flux reduction is limited by concentration polarisation. Such plant often runs for long periods without the need for intermediate cleaning.

Single-pass, multistage designs tend to be favoured for large-scale plants that utilise modules with relatively high water recovery factors, such as spiral and hollow-fibre systems, which are widely used. Brackish water plants usually operate at high water recovery rates (70–95%), so there is a considerable increase in retentate composition toward the end of the process. This gives rise to an increase in the amount of salt which permeates toward the end of the process, so the reduction in solids is not as high as may be expected. Energy costs are about 1–1.5 kWh/m^3 permeate. There are many installations processing in excess of 10 000 m^3/d. Retentate concentrations are usually kept to below 10 000 ppm, to avoid high osmotic pressures and prevent fouling due to mineral scale.

However, more recently the problems involved in treating seawater have been overcome with new membranes that resist compaction at high pressures (up to 70 bar) and show high salt rejections. Some of the earlier installations had a two-stage unit, with some of the permeate being treated in a brackish water type installation. This permeate was then combined with untreated permeate from the first stage to produce an overall product. However, with improvement in membrane performance, it is possible to produce potable water from single-stage processes. Usually seawater is reduced from about

35 000 ppm dissolved solids to about 500 ppm. Water recovery rates are much lower than for brackish water treatment (25–30%) and energy requirements are higher (5.6–7.6 kWh/m^3).

Reverse osmosis has been used in a wide range of water purification processes, as well as water recovery. Nanofiltration membranes were found to be capable of rejecting a range of synthetic organic compounds from water, although some of the lower molecular weight components evaluated, such as ethylene dibromide, were more permeable. However, a mass balance suggested that some of the higher molecular weight components were adsorbed onto the membrane surface (Duranceau *et al.*, 1992). They have also been investigated for removing pesticides and components responsible for the colour, from ground water, as well as for purifying water for carbonation and soft drinks. For high-grade purity water production, for analytical purposes, it may be treated by double reverse osmosis, as mentioned earlier.

Further information on the performance of systems and the economics of the process are given by Gutman (1987) and Nishimura and Koyama (1992).

If lower total solids are required, the permeate can be subjected to a second process, known as double reverse osmosis. This has moved on to the production of ultrapure water for the preparation of microelectronic components and uses in medical laboratories and the pharmaceutical industries. The scheme for the production of ultrapure water is given by Gutman (1987) and Nishimura and Koyama (1992). More information is also given in Chapter 1.

3.9.3 Milk processing

The potentialities for processing milk by reverse osmosis are not as great as those for ultrafiltration (Grandison and Glover, 1994). It can be used for concentrating full cream milk up to a factor of 2–3 times. Flux decline is similar to that for ultrafiltration, showing a linear relationship when flux is plotted against the log of the concentration factor. Flux rates for skim milk are only marginally higher than those for full-cream milk. Recorded flux rates at the start of the process are up to 40 l m^{-2} h^{-1}.

Factors affecting the flux rate are similar to ultrafiltration. The product concentration attainable is nowhere near as high as that for evaporation, due to increasing osmotic pressure and fouling, due mainly to the increase in calcium phosphate, which precipitates out in the pores of the membrane. Therefore most of the commercial applications have been for increasing the capacity of evaporation plant.

Other possible applications that have been investigated and discussed include: the concentration of milk on the farm for reducing transportation costs; for yoghurt production at a concentration factor of about 1.5, to avoid addition of skim-milk powder; for ice-cream making, also to reduce the use of expensive skim-milk powder; for cheese-making to increase the capacity of the cheese vats, and for recovering rinse water. Whey can also be concentrated, to reduce transportation costs or prior to drying. Flux values for sweet whey are higher than for acid whey, which in turn are higher than for milk, for all systems tested (Glover, 1985). The main reason for differences between acid whey and sweet whey is believed to be the much higher levels of calcium in acid whey, which acts as a foulant. Whey can be concentrated from 6% to 24% solids, at as low as 7°C. A typical plant (PCI technical literature) has a membrane surface area of 327 m^2, in three

stages, with each stage having 42 modules. Power consumption is 78 kW, the feed rate is 60 m^3 h^{-1} and the temperature is 28°C. Under these conditions the membrane lifetime is three years. Suarez *et al.* (1992) measured the mass-transfer coefficient (k) and the membrane concentration c_m at different operating pressures and pH values in the range 5.1 to 9.0. They found that both k and c_m increase with increasing differential pressure. At high differential pressures, convective flow was high and it was suggested that the process was not mass transfer controlled.

Pal and Cheryan (1987) reported some success for using RO concentrated milk (31% TS) for khoa manufacture, with the potential for large savings in energy. However, the average flux was reported as only 8.1 l m^{-2} h^{-1} at 30°C.

Grandison and Glover (1994) reported that for all practical purposes all the components of milk are retained by the membrane and only a small proportion of the smallest ions escape. Rejections of the whole mineral content of milk greater than 99% are reported with rejections of Na^+ of 99%, K^+ of 98% and Cl^- of 94%. From a detailed study (Morales *et al.*, 1990), it was found that different membranes and membrane configurations can influence both flux and rejection of components during milk and whey processing. They also found that total solids rejection was independent of temperature and was higher when milk, rather than whey, was processed. In general, all the membranes were capable of rejecting 100% of the true protein. Rejection of non-protein nitrogen, lactose and total BOD was affected by change in the operating conditions, type of feedstock and type of membrane employed, whereas rejection of ash was substantially insensitive to variations in operating conditions and changes in feedstock.

Milk concentrate is thus not likely to have the same extent of heat damage as that produced by evaporation. It may also be slightly different in composition, which may affect the texture and stability of products derived from it. For example the storage stability of RO concentrate has been found to be better than that produced by evaporation. However, this may not be the case for all membranes and flow geometries and will depend upon the rejection for the different components.

Fouling is a major problem and the main component of the fouling layer is usually found to be protein. Kulozik and Kessler (1988a) considered that there were two resistances to permeation, one due to laminar flow through the deposit and the other due to transport by diffusion through the membrane. They found that the inorganic ions in milk increased the resistance offered by the deposited layer. However, the stability of the deposit and the ease at which it is removed by rinsing is dependent upon the low molecular weight components, particularly calcium (Kulozik and Kessler, 1988b). In sweet-whey processing, fouling resulting from calcium phosphate is a problem at higher concentrations. This can be reduced by prepasteurisation, causing some precipitation of the calcium phosphate or by reduction of pH, using acid or addition of carbon dioxide, where 0.9 mg l^{-1} has been recommended. One suggestion to reduce the flux decline due to fouling is to control it by use of time-dependent settings of pressure and flow velocity, rather than constant values (Boxtel and von Otten, 1992).

Some uses for RO in the dairy, as a water source, are reviewed by the International Dairy Federation (International Dairy Federation, 1988).

Nanofiltration has been used for partially reducing calcium and other salts in milk and whey, with typical retention values of 95% for lactose and less than 50% for salts. Guu

and Zall (1992) have reported that permeate subject to nanofiltration gave improved lactose crystallisation. NF provides much greater potential for influencing the heat stability of the milk.

Reviews of the use of RO and UF in dairying applications include El-Gazzar and Marth (1991), and Renner and El Salam (1991).

3.9.4 Fruit and vegetable juices

Reverse osmosis has found application in the processing of fruit and vegetable juices, sometimes in combination with ultrafiltration and microfiltration. The osmotic pressure of juices is considerably higher than that for milk. There has been a dramatic increase in fruit juice consumption; most juice needs to be concentrated prior to freezing and is then transported frozen.

It is advantageous to minimise thermal reactions, such as browning, and to reduce loss of volatiles. From a practical viewpoint, the flux rate and rejection of volatiles is important. RO modules can cope with single-strength clear or cloudy juices and also fruit pulp. RO can be used to produce a final product, as in the case of tomato paste and fruit purees, or to partially concentrate, prior to evaporation.

RO is a well-established process for concentrating tomato juice from about 4.5 Brix, to between 8 and 12 Brix. Plant capacity of some commercial units ranges between 25 and 37 $m^3 h^{-1}$ and the power consumption is 175 kW. Such plants run at inlet pressures of 50 bar and between 60 and 70°C. The modules are placed in parallel, due to the high viscosity of the product. Tomato juice has been reported to have been concentrated to over 20%, but special care is needed because of the high viscosity (Lafferty, 1992).

Other fruit juices which have been successfully concentrated are apple, pear, peach and apricot. Where juices have been clarified, osmotic pressure limits the extent of concentration and up to 25 Brix can be achieved. Unclarified juices may be susceptible to fouling. With purees and pulps, the viscosity may be the limiting factor and these can be concentrated to a maximum of 1.5 times. Gherardi *et al.* (1989) have used combined UF and RO of pear and peach purees, with the UF permeate being further concentrated by RO, and measured the partitioning of volatile and non-volatile flavour components between the different fractions. Studies by Bowden and Isaacs (1989) have indicated that cloudy pineapple juice can be concentrated by reverse osmosis from about 13 to 25% soluble solids with good quality retention. Losses of soluble components into the permeate were found to be very slight.

It has also been found useful to recover solids from diluted juice, waste juice and washwater or from depectinised unclarified juice (6–22 Brix). Sheu (1987) reported that the most energy-efficient process for production of apple juice concentrate at 72 degrees Brix, involves processing the clarified juice from ultrafiltration to 21 degrees Brix by reverse osmosis, prior to evaporation. Chou *et al.* (1991) have studied the loss of flavour compounds during the concentration of apple juice in more detail and concluded that low temperatures (20°C) and high pressures were the most effective for reducing losses. Rejection of flavour compounds was higher using polyamide than polyether–urea thin-film composite membranes.

Citrus juices are also concentrated. For oranges, the high hesperin content of the juice results in fouling and rapid flux decline. Performance is improved by pasteurisation and

filtration to below 0.4 mm. Processing temperatures are between 20 and 40°C and the fouling is reduced by a 10 minute flush with 0.25% caustic soda every six hours. Pectin also contributes to fouling and pectinase treatment has been found to prevent fouling. A process has been described for concentrating orange juice up to 42 Brix, by a combination of UF and RO (Cross, 1989). However, relatively little work has been done on other citrus juices.

Braddock *et al.* (1991) concentrated citrus juice essences containing between 2 and 20% ethanol. There was a substantial reduction in the rejection of ethanol during the concentration of citrus juice essences, which was 90% at 2% alcohol to 40% at 31% alcohol. Acetaldehyde rejection decreased from 65% down to 25% over the same range. Rejection for larger aroma molecules, such as ethyl butyrate, hexanal and terpenes, was generally greater than 85%. The flux rate at 8.3 MPa fell by more than 10 times during concentration from 0.01% to 30% alcohol and flux declined in a linear manner, with the log of the alcohol content.

Vegetable juice processing has received some attention, although the market is nowhere near as large as that for fruit juices. Koseoglu *et al.* (1991b) present data for celery, tomatoes, carrots and cucumbers. The macerated vegetables are pressed and the screened juice is subject to ultrafiltration. The clear permeate can then be concentrated by reverse osmosis and added back to the retentate from ultrafiltration. For all vegetables the flux rates for reverse osmosis were lower than for ultrafiltration and were as follows ($l\ m^{-2}\ d^{-1}$): carrot, 30.5; celery, 82.5; tomato, 295.7 and cucumber, 432.0.

3.9.5 Other applications

Thin-film composite membranes have been assessed for sugar cane and beet juice concentration, up to 80°C and pressures between 40 and 80 bar, Kosikowski (1986). Prefiltration or clarification to remove soil and fibre is essential. Polysulphone membranes perform better than cellulose acetate. However, it was pointed out that the potential advantages over evaporation are not so great as there is always plenty of surplus steam available in sugar factories.

Instant coffee is a very popular beverage and it is possible to concentrate the coffee extract from about 13% to 36% total solids at 70°C, with little loss of solids. Thin-film composite membranes have been found to give a better retention of aromatics. The concentrate is then evaporated to about 48% solids, prior to drying. Currently, instant tea is also being heavily marketed and reverse osmosis has been investigated for preconcentration. However, much of the research is done within the private sector. From our experience, the tannin components in these drinks are likely to cause fouling problems.

Koseoglu *et al.* (1991a) have provided a comprehensive review on the application of membrane processes in cereal processing. There is more scope for ultrafiltration, although reverse osmosis is used in combination with ultrafiltration for recovery of protein and other solids from thin stillage materials, which are the remnants after distilling the alcohol (Wu, 1987). Again, cellulose acetate membranes were found to be more permeable than polyamide ones. Reverse osmosis is also used for waste recovery and more efficient use of processing water in corn wet-milling processes. More detail is provided by Wu (1988). The process of counter-current reverse osmosis (CCRO) is

described, which allows concentration to 30% TS, rather than the 20% by normal reverse osmosis. This is achieved by passing a 30% solution on the permeate side of the membrane in a counter-current direction to the retentate, thereby reducing the osmotic driving force. A more recent development is the introduction of membranes for concentration of dilute sweetwaters up to 60 Brix, without the use of excessive pressures.

Eggs can be concentrated prior to drying. Commercial plant is available for concentrating egg white, to about 20% solids. In one particular application egg white is concentrated and dried, after lysozyme has been extracted. It is not crucial to concentrate egg yolk, which contains about 50% solids. Egg processing wastes can also be treated, to reduce BOD, recover solids for animal feed and reutilise water (Roberts, 1989).

Poor wine is usually produced from grape juice (must) containing less than 17% sugar. Production of wine from must concentrated slightly by reverse osmosis is improved compared to that produced by adding sugar, although the costs are likely to be higher.

Reverse osmosis has been reported to remove some of the compounds responsible for the 'old' flavour of wine. It is usually superior to wine produced from evaporated must.

Dealcoholisation is an interesting application, using membranes which are permeable to alcohol and water. In a process akin to diafiltration, water is added back to the concentrated product, to replace the water and alcohol removed in the permeate. Such technology has been used for the production of low or reduced alcohol, beers, ciders and wine. It can be applied either as a single process, using a feed and bleed system, or as a two-stage process, where the concentrate from the first stage is rediluted with water and subjected to a second reverse osmosis process. For these applications, cellulose acetate membranes are used rather than the thin-film composites, because their rejection values for ethanol are lower. Gutman (1987) reported that the removal efficiency (rejection) of ethanol was 12% for cellulose acetate membranes and 28% for polyamide membranes. More detailed information on the rejection characteristics and flux data for ethanol/water systems for cellulose acetate, polyamide and other membranes has been collated by Leeper (1986). Ethanol rejections for cellulose acetate ranged between 1.5 and 40%; for polyamides between 32.8% and 60.9% and for other hybrid membranes, as high as 91.8%. Further information on the quantitative aspects of diafiltration in terms of how yields and diafiltration volumes are affected by these different rejection values, are provided in Section 4.4. Some methods for producing low-alcohol wines of approximately 5% are described, Chinaud *et al.* (1991). For beer, quality can be maintained by adding carbon dioxide while it is being concentrated. The use of membranes which reject alcohol could also be used for concentrating beer or fortifying wines. It is also possible to produce low alcohol beverages by use of liquid membranes or using pervaporation (Leeper, 1986).

McGregor (1989), has examined the use of a high-flux thin-film composite membrane for concentration of L-phenylalanine from clarified bioreactor harvest media. The rejection coefficient was found to decrease as the retentate concentration increased, and a concentration of 100 g/l could be obtained. Flux rates were recorded between 17 and 119 l m^{-2} h^{-1}. A cascade system could be used to recover almost all the phenylalanine. The author concluded that this study shows the importance of empirical evaluation as the basis of design.

In many cases, combinations of processes are used, some of which are discussed in later chapters. One interesting application is the use of formed-in-place membranes (FIP), where the membrane is made by deposition of either inorganic solutes, Thomas *et al.* (1992) or organic polymers within the matrix of a porous tube (Spencer and Thomas, 1991). Mechanisms of fouling, a cleaning regime and rejuvenation of FIP membranes are described.

SYMBOLS

c	concentration
d	pore diameter
f	concentration factor
g	acceleration due to gravity
h	head
i	degree of ionisation
J	flux
L	pore length
M	molecular weight
m^1	mass flow rate
P	pressure
ΔP	pressure drop
Q	volumetric flow rate
R	rejection
R	gas constant
T	absolute temperature
V	volume
V_m	partial molar volume
VRF	volume reduction factor
W	power utilisation

Greek symbols

γ	activity coefficient
π	osmotic pressure
μ	viscosity
θ	contact angle

Subscripts

F	feed
c	concentrate
p	permeate
s	solute
w	water
m	membrane
g	gel

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Chapter 4

Ultrafiltration

M. J. LEWIS, Department of Food Science and Technology, The University of Reading, RG6 6AP.

4.1 INTRODUCTION

Ultrafiltration offers the opportunity to concentrate large molecular weight components without the application of heat or a change of phase. Such components are rejected by the membrane, whereas the permeate produced will contain the low molecular weight components present in the food, at a concentration similar to that in the feed. This results in an increase in their concentration both on a wet weight and dry weight basis in the solution. It is a pressure-activated process, with pressures in the range of 1–15 bar; these pressures are considerably lower than those used in reverse osmosis. For many heat labile macromolecules, e.g. proteins and starches, concentration by UF at ambient temperature will minimise heat-induced reactions which may adversely influence their functional behaviour in foods. Some important functional properties are solubility, foaming capacity, gelation, emulsification capacity, fat and water binding properties. These are discussed in more detail in Section 4.5.

In the case of enzymes or pharmaceutical agents, their biological activity needs to be conserved. It also affords the opportunity to separate small molecular weight components from complex mixtures, containing components with a wide range of molecular weights. There have also been investigations into using UF for protein fractionation, but this is not straightforward due to the diffuse nature of the membranes and their selectivity.

UF is also very useful for recovering valuable components from food processing waste streams and fermentation broths. Probably the greatest impetus has come from the dairy industry and dairying applications. However, in all applications, flux decline due to concentration polarisation and fouling are probably the two most important practical aspects.

4.2 PROCESSING CHARACTERISTICS

This section will deal with some of the important processing parameters encountered in ultrafiltration. There are various factors which will influence the outcome of the process, such as the concentration factor and rejection. See Section 3.3.

The extent of the concentration is defined by the concentration factor (f), defined as V_F/V_c (see eq. (3.5)). Usually the permeate is the biggest fraction by volume. Milk for cheese making is concentrated by UF fivefold, whereas cheese whey is concentrated twentyfold for the production of protein concentrates. Sometimes the resulting permeates are further concentrated by reverse osmosis.

4.2.1 Rejection or retention factors

The rejection or retention factor (R) of any component is defined as

$$R = (c_F - c_P)/c_F \tag{4.1}$$

where c_F is the concentration of component in the feed and c_P is the concentration in the permeate.

The rejection is determined experimentally for each component in the feed, by sampling the feed and permeate at the same time and analysing that component. It is very important and will influence the extent (quality) of the separation achievable.

Rejection values normally range between 0 and 1; sometimes they are expressed as percentages (0 to 100%).

when $c_P = 0$; $R = 1$; all the component is retained in the feed

when $c_P = c_F$ $R = 0$; the component is freely permeating.

In ultrafiltration experiments, some workers have measured negative rejection, i.e. $c_P > c_F$, particularly for minerals. It is not immediately obvious why this should have occurred. Possible explanations for this are higher concentrations at the membrane surface than in the bulk, due to concentration polarisation. However, this is unlikely to be the case for freely permeating species. Another explanation is the basis on which concentration is measured (Glover, 1985). This may arise when there is substantial fat in the feed which is rejected by the material. It is suggested that concentrations be expressed in the aqueous portion. A third explanation lies in the Donnan effect; Donnan predicted and later demonstrated that concentration of electrolyte in the solutions on either side of a dialysis membrane were unequal when the colloid on one side was electrically charged (see later). For example, at low pH values, where proteins are likely to be positively charged, this could lead to higher concentrations of cations in the permeate.

Rejection characteristics can readily be determined for different substances using different membranes. This is one practical way of selecting the most appropriate membrane for a particular application. Rejection values may also be influenced by operating conditions.

An 'ideal' ultrafiltration membrane would have a rejection value of 1.0 for high molecular weight components and zero for low molecular weight components. However, typical values observed for real membranes are between 0.9 and 1.0 for high molecular weights and between 0 and 0.1 for low molecular weight components. Values for

minerals often are usually in the region of 0.1, but may be as high as 0.5, if the mineral binds to macromolecules. *It is important to appreciate that any component with a rejection value greater than 0 will increase in concentration during the course of an ultrafiltration process.* Rejection values can be used to check the integrity and performance of a membrane. Some values for components in dairy processing are given in Table 4.1. Note the relatively high values for minerals, which suggests some binding to the proteins, particularly for calcium and magnesium. Membrane manufacturers sometimes present performance data in terms of rejection values of a range of components of different molecular weights (see Table 4.2). This will give some guidelines in terms of selection. However, very rarely are those components selected that one is interested in. An alternative form of representation widely used is the *molecular weight cut-off* value.

Table 4.1. Rejection characteristics obtained during ultrafiltration of dairy products

Product	Protein[a]	Lactose	Ash
Sweet whey	0.85–1.0	0–0.2	0–0.5
Acid whey	0.85–1.0	0–0.2	0–0.5
Skim milk	0.965–1.0	0–0.2	0–0.5
Whole milk	0.965–0.999	0–0.03	0–0.1

a Based on Kjeldahl nitrogen × 6.38.
Taken from Lewis (1982).

Table 4.2. Some cited rejection characteristics for different components

	MW	3000	10 000	30 000	100 000
Insulin	6 000	>0.98	—	—	—
Cytochrome C	12 400	>0.98	0.85	0.45	—
a-Chymotrypsinogen	24 500	>0.98	0.95	0.75	0.20
Albumin	67 000	>0.98	>0.98	0.95	0.30

Adapted from data from Amicon (1992).

The molecular weight cut-off values for UF membranes range between about 2000 and 300 000. At values of about 2000, it overlaps with nanofiltration or 'loose reverse osmosis', whereas at 30 000 it overlaps with microfiltration. Generally the applied pressure required will decrease with increasing cut-off value and pressures in the range 1–15 bar are used.

It is implied that a membrane with a molecular weight cut-off of 5000, would reject all components with that molecular weight value or higher ($R = 1$) and allow components below that molecular weight to permeate freely. Often dextrins have been used for estimating molecular weight cut-off, but these are linear molecules. However, due to the

diffuse nature of the membrane, this is not so. This approach ignores molecular shape, charge and any interactions between components in the feed, and at the membrane surface and within the membrane itself. Nevertheless it is useful for a preliminary (initial) selection of a suitable membrane. However, it tells you nothing about the rejection value of a component below the molecular weight cut-off, say 500 or 1000. In fact, it rather implies that such components would be freely permeating. In reality, this is not the case as most membranes are diffuse in their separation ability. The concept of a sharp and diffuse membrane is useful in this respect (see data from Table 4.2).

Figure 4.1 shows the rejection characteristics of two such membranes. The sharp membrane is an ideal situation, offering the perfect separation. Real membranes offer quite diffuse rejection characteristics, requiring a molecular weight difference of about tenfold to provide an effective separation. Therefore they would give a poor separation of components with slight differences in molecular weights, even components with differences up to two times would not necessarily be well separated. For example it would not be easy to fractionate the proteins in cheese whey or to separate monosaccharides from disaccharides. McGregor (1986) has undertaken some interesting experiments, using electrophoresis to examine the sharpness of separations performed on mixtures of protein of different molecular weights. His results showed considerable differences in the sharpness of the separation between different membranes with the same nominal molecular weight cut-off value. Gekas *et al.* (1990) found that experimental flux and rejection data correlated better with porosimetric data (pore size and pore size distribution as measured by bubble pressure and solvent permeabilities) than molecular weight cut-off value.

These types of observation illustrate that although some physicochemical measurements might be useful, the selection of the best membrane is best done experimentally, by measuring the rejection characteristics of the components to be separated at the selected operating conditions.

There is also evidence that the rejection value for most components increases during the course of an ultrafiltration process. Some of the experimental work on rejection measurement and practical problems involved are described in Section 4.2.4.

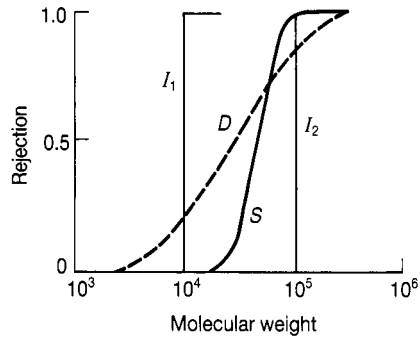

Fig. 4.1. Characteristics of a sharp and diffuse membrane: I_1, ideal, 10 000 molecular weight cut-off: I_2, ideal, 100 000 molecular weight cut-off; S, sharp membrane; D, diffuse membrane.

4.2.2 Yield

Ultrafiltration is now being used to concentrate and recover some very valuable compounds. The yield or recovery of a component is a very important variable, as it will strongly influence the economics of the process.

The *yield* of a component is defined as the fraction of that component, originally present in the feed, which is retained in the concentrate. For recovery of components it is important to have a high yield. However, when washing out components, such as toxins, the yield should be low.

For a batch process, it can be shown that the yield of any component depends upon the concentration factor and rejection.

Concentration factor

$$(f) = V_F/V_C \tag{4.2}$$

and the yield (Y) is given by

$$Y = \frac{\text{mass component in final concentrate}}{\text{mass substance in feed}} = \frac{V_C c_C}{V_F c_F} \tag{4.3}$$

where V_C and V_F are the volumes of feed and concentrate and c_C and c_F are the concentrations in the concentrate and feed.

If we now consider a batch concentration process depicted by Fig. 4.2, where permeate is removed and the retentate is recycled:

> At any instance let the volume of the concentrate = V and the concentration of the component of interest = c

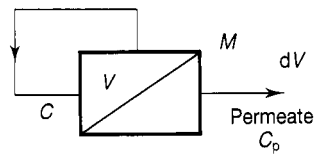

Fig. 4.2. Batch concentration process for yield calculation.

Let the removal of a small volume of permeate (dV), result in a change of concentration (dc).

A mass balance on the component will give the following equation:

$$Vc = (V - dV)(c - dc) + c_P\, dV$$

(feed) (concentrate) (permeate)

Rejection

$$(R) = (c - c_P)/c$$

Thus

$$c_P = c(1 - R)$$

Eliminating c_P gives

$$Vc = (V - dV)(c - dc) + c(1 - R)\, dV$$
$$- V\, dc = cR\, dV$$

(*Note*: $dV dc$ is assumed to be negligible.)

$$-\int \frac{dV}{V} = \int_{c_F}^{c_C} \frac{dc}{c} \frac{1}{R}$$

Integration between the final and initial conditions gives:

$$\ln\left(V_F/V_C\right) = 1/R \ln\left(c_C/c_F\right) \tag{4.4}$$

If $\ln (V_F/V_C)$ is plotted against $\ln (c_C/c_F)$, the gradient is $1/R$.

$$V_F/V_C = f = \left(c_C/c_F\right)^{1/R} \tag{4.5}$$

From eqs. (4.2) and (4.3), it can be shown that

$$c_C/c_F = Yf$$

Substitution into eq. (4.5) gives

$$V_F/V_C = (fY)^{1/R}$$

Therefore $f = (fY)^{1/R}$. This simplifies to $Y = f^{R-1}$. Therefore the yield

$$Y = f^{R-1} \tag{4.6}$$

However, this equation applies only if the rejection remains constant. Nevertheless, it is extremely useful, as it gives an insight into the features of the separation process. Let us consider the two extreme values of rejection:

If $R = 1$, then yield = 1; all the material is recovered in the concentrate.

If $R = 0$, then yield = $1/f$; in this case the yield is determined by the concentration factor. As the concentration factor is finite (typically 2–20), the yield can never be zero; i.e. it is not possible to remove all of a component from a feed by ultrafiltration alone. Diafiltration may be more useful in helping to achieve this objective (see Section 4.4).

However, for most components being concentrated, the rejection values are close to 1.0, typically 0.9–1.0, whereas for those being removed the values would be between 0 and 0.1.

Table 4.3 shows a range of yield values for some different concentration factors. One interesting point is that losses can be quite high, even though the rejection value appears good; e.g. for $R = 0.95$ and a concentration factor of 20, the yield is 0.86. Therefore 14%

Table 4.3. Yield values for different concentration factors and rejections

Concentration factor	Rejection						
	0	0.1	0.2	0.5	0.9	0.95	1.00
2	0.50	0.54	0.57	0.71	0.93	0.97	1.0
5	0.20	0.24	0.28	0.45	0.85	0.92	1.0
10	0.10	0.13	0.16	0.32	0.79	0.89	1.0
20	0.05	0.07	0.09	0.22	0.74	0.86	1.0
50	0.02	0.03	0.04	0.14	0.68	0.82	1.0

of the component is lost in the permeate. Yield values are also sometimes quoted as percentages.

However, this equation gives the maximum yield, which would be for a batch process. The yield is likely to be lower for a continuous single or multistage process, simply because steady state is achieved at higher levels of concentration. For such a process the yield is given by

$$Y = \frac{1}{f - R(f-1)}$$

The concentration of a component in the final resulting concentrate (c_C) can be calculated from the following equation:

$$c_C = c_F Yf \tag{4.7}$$

or

$$c_C = c_F f^R \tag{4.8}$$

However, there is some evidence that rejection does not remain constant. During a batch ultrafiltration experiment the rejection of most components rises, as has been observed on many occasions (see Fig. 4.3).

4.2.3 Average rejection

In situations where the rejection does change significantly, an alternative evaluation procedure is to measure the yield for the process, and then to work backwards to calculate the rejection value, which would have given rise to that yield. This rejection value is termed the average rejection value (R_{av})

$$Y = \frac{V_C c_C}{V_F c_F} = \frac{c_C}{c_F}\frac{1}{f} \tag{4.9}$$

If this expression for yield is equated with that from eq. (4.6):

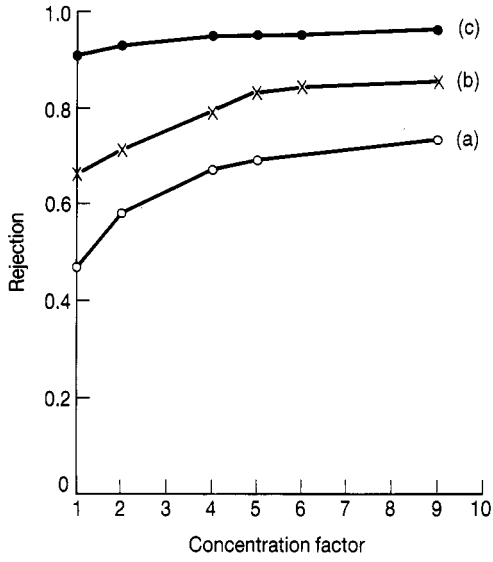

Fig. 4.3. Change in rejection during UF: (a) glucosinolates; (b) total solids; (c) protein.

$$f^{R-1} = (c_C/c_F)/f$$

$$f^R = c_C/c_F$$

$$R \log f = \log(c_C/c_F)$$

$$R = \log(c_C/c_F)/\log f \tag{4.10}$$

This expression for the rejection is effectively an average rejection (R_{av}) for the process. Therefore:

$$R_{av} = \log(c_C/c_F)/\log f \tag{4.11}$$

Estimation of average rejection is based upon knowing the initial and final concentrations and the concentration factor.

It is interesting to note that, in this case, the membrane rejection can be determined without sampling the permeate.

Therefore the average rejection is defined as the rejection value which would provide the same yield which was actually found in the process, even though the instantaneous rejection may have been changing throughout.

4.2.4 Practical rejection data

Although some of the proposed models predict how rejection will be influenced by operating conditions and pH, there is often little agreement between theory and practice

for most food systems. Therefore it is very important to measure rejection data under the prevailing operating conditions.

Lewis (1982) has compiled rejection data for different systems. It was not always clear whether rejection data for proteins was based upon crude protein or true protein. Ultrafiltration could be useful for removing non-protein nitrogen. There also appeared to be some confusion between the terms rejection and yield in some of the earlier reports.

Table 4.1 shows some rejection data for some dairy products, reported by the International Dairy Federation (1979).

Figure 4.3 shows rejection data taken during the batch ultrafiltration process during concentration of rapeseed meal, for crude protein, total solids and glucosinolates. For all components, there is an increase in rejection as concentration proceeds, with the increase being most marked between concentration factors of 1 and 2. Many investigators have reported similar increases in rejection as concentration proceeds.

Table 4.4 shows some data for the average rejection data for proteins and glucosinolate, extracted at different pH values, determined by the method above. Yield values are also presented. In such complex systems the performance is also strongly affected by pH (see Sections 4.3.2 and 4.5.2).

Table 4.4. Average rejection (R_{av}) and yield values for glucosinolates and crude protein during batch ultrafiltration processes at different pH values.

pH	Glucosinolate	Crude protein
2.5	0.50 (0.45)	0.97 (0.95)
3.5	0.39 (0.38)	0.93 (0.89)
7.0	0.28 (0.31)	0.81 (0.74)
9.0	0.36 (0.36)	0.95 (0.92)
11.0	0.44 (0.41)	0.85 (0.92)

Yield values in brackets.
Glucosinolates are expressed as isothiocyanates.

Therefore, rejection values are very important as they influence the nature of the separation obtained, as well as the yield (or loss) of components. These aspects assume greater importance as the value of the product increases. Changes in rejection during a process could also be indicative of some important changes taking place at the surface of the membrane. The effects of pressure and temperature on rejection, as predicted by some of the models, are discussed in Chapter 3. Some practical problems associated with UF of proteins, such as adsorption and pH effects, are described by Sirkar and Prasad (1987).

4.3 PERFORMANCE OF ULTRAFILTRATION SYSTEMS

Permeate flux

In UF process applications, the two most important parameters are the membrane

rejection (see also Chapter 3) and the flow rate of permeate or permeate flux, hereafter abbreviated to 'flux'. The flux will probably be measured in gallons/min or litres/hour, but it is usually presented in terms of volume per unit time per unit area ($l\ m^{-2}\ h^{-1}$). Expressed this way it allows a ready comparison of the performance of different membrane configurations with different surface areas. Flux values may be as low as 5 or as high as $450\ l\ m^{-2}\ h^{-1}$. The flux is one of the major factors influencing the viability of many processes.

UF processes have been subject to a number of modelling processes, in an attempt to predict flux rates and rejection values from the physical properties of the solution, the membrane characteristics and the hydrodynamics of the flow situation, in order to optimise the performance of the process.

4.3.1 Transport phenomena and concentration polarisation

Ultrafiltration is usually regarded as a sieving process and in this sense the mechanisms are simpler than for RO. However, it is important to remember that for pressure-driven membrane processes, the separation takes place not in the bulk of solution, but in a very small region close to the membrane, known as the boundary layer, as well as over the membrane itself. This gives rise to the phenomenon of concentration polarisation over the boundary layer. (Note that in streamline flow the whole of the fluid will behave as a boundary layer.)

Concentration polarisation occurs whenever a component is rejected by the membrane. As a result, there is an increase in the concentration of that component at the membrane surface, and a concentration gradient over the boundary layer. This increase in concentration offers a very significant additional resistance, and for macromolecules may also give rise to the formation of a gelled or fouling layer on the surface of the membrane (see Fig. 3.4). It is interesting to note that the boundary layer does not establish itself immediately at the point where the fluid first contacts the membrane. Rather it takes some distance for it to be fully established. This distance taken for it to be fully established has been defined as the entry length, and the process of establishment is illustrated for a tubular membrane in Fig. 4.4. Howell *et al.* (1990) have analysed flux conditions over the entry length and have concluded that the flux and wall concentrations change quite considerably over the developing boundary layer, although changes were less marked for a fouled membrane. There would also be less likelihood of operating in the pressure-independent

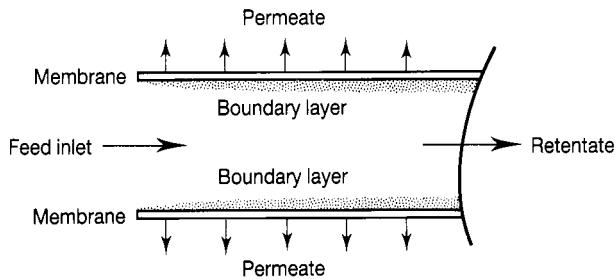

Fig. 4.4. Development of the concentration polarisation or boundary layer.

region in the entry length, so there could be an improvement to the flux by use of higher pressures. They reported that the entrance length may be greater than 1 m, but that most of the benefits to flux which could occur using higher pressures would be over the first 20 cm.

One much used model considers a number of resistances in series. Therefore, during the transfer of components from the bulk of the solution to the permeate, the main resistances are due to the membrane (R_m), the fouling layer (R_f) and the polarisation layer (R_p).

Therefore the flux can be expressed as

$$J = \Delta P / \mu (R_m + R_f + R_p) \tag{4.12}$$

where μ is the viscosity of the solvent. The pressure term may be modified to $(\Delta P - \Delta \pi)$, to account for differences in osmotic pressure, but in most UF applications, the osmotic pressure differences ($\Delta \pi$) are negligible.

This type of model is sometimes known as the 'resistance in series model'. In practical terms, the effects of concentration polarisation can easily be seen, as there is a marked reduction in flux, when water is replaced by the solution to be ultrafiltered, using either a dynamic or static start (Fig. 4.5). For a new membrane, flux data is determined using water, before use, as this provides an indication of the condition of the original membrane and its resistance (R_m), or a membrane after cleaning. Membrane-cleaning protocols are designed to restore the water flux back to its original value. As soon as the water is replaced by the fluid, the flux rate will fall by a factor of 2–10 times, in a very short period of time, usually less than one minute. Thus the equilibrium described is achieved in a relatively short period of time. As concentration proceeds, the flux will further decline, due to a combination of an increase in the viscosity (total solids) and the process of fouling. However, it is not easy to separate the effects of polarisation, fouling and concentration increases. Therefore, experiments to observe the effects of fouling are usually performed at constant composition, by returning the permeate to the feed tank.

Also, it is not straightforward to assess the individual contributions of fouling and concentration polarisation to the flux decline during the initial transition from water to product. Some fouling is evident with most systems and is assessed by the decline in flux

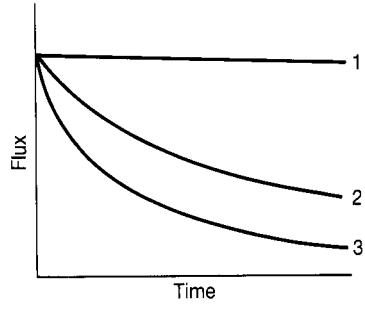

Fig. 4.5. Flux decline due to concentration polarisation and fouling: (1) water flux; (2) moderate flux decline; (3) rapid flux decline.

rate with time, usually for a period of 30–120 min, at constant composition. Fouling will be discussed in greater detail later.

In the absence of fouling, there are two main transport steps: (1) through the boundary (polarisation layer) and (2) through the membrane (Aimar, 1987). The first depends upon the hydrodynamics, on flow rate of solvent to the membrane (permeate flux), fluid composition and transport properties. The second depends upon the applied pressure and the properties of the membrane, namely its average pore size, distribution of pore size and its chemical properties. One of these transport steps is likely to be the rate limiting process.

In general terms, the effects of operating parameters are shown in Fig 4.6. It should be noted that results are obtained for a constant composition. As the concentration of the feed increases, the flux will further decline. It can be seen that there is a pressure-dependent region (*AB*) and a pressure-independent region. However as the flux rate increases, the rejected materials will increase in concentration and concentration polarisation becomes limiting. In this region flux rates can be increased by higher flow rates and operating temperatures. Therefore this resistance approach provides an explanation for the observed occurrence of pressure-dependent and pressure-independent regimes. At low pressures and low flux rates, the membrane offers the controlling resistance. This would suggest that ultrafiltration processes are best operated at pressures corresponding to the initial onset of the pressure-independent region. Use of higher pressures would only be wasteful of energy. There is some evidence that this could also give rise to a decrease in the flux, due to compaction of the membrane or fouling layer.

In the pressure-independent region, the following analysis has been performed to model the flux performance, based upon a material balance at the membrane surface. This is known as the film theory model (see Fig. 4.7).

A dynamic equilibrium is established, where the convective flow of the component to the membrane surface equals the flow of material away from the surface, either in the permeate or back into the bulk of the solution by diffusion, due to the concentration gradient established. This is expressed as

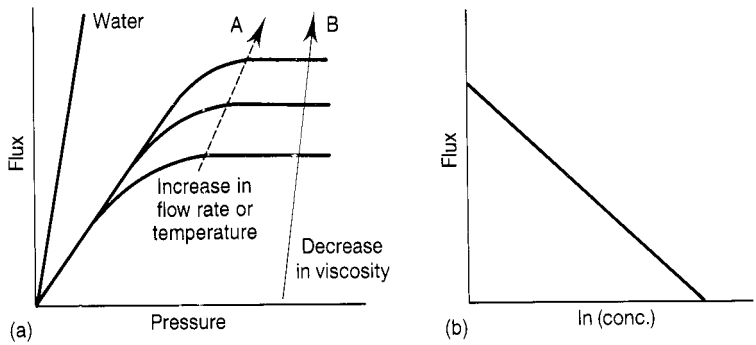

Fig. 4.6. (a) Effects of operating pressure and flow rate on flux; (b) flux decline measured against concentration factor.

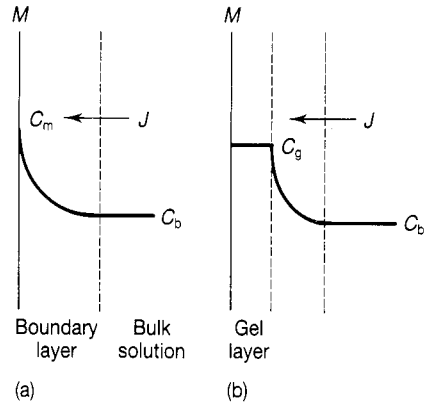

Fig. 4.7. Concentration polarisation: (a) without a gel layer; (b) with a gel layer.

$$Jc = D\ \mathrm{d}c/\mathrm{d}y + Jc_P$$

$$J(c - c_P) = D\ \mathrm{d}c/\mathrm{d}y$$

Integration over the boundary layer of thickness L gives

$$J = D/L\ \ln\left[(c_m - c_P)/(c_b - c_P)\right] \tag{4.13}$$

where D = diffusion coefficient; L = boundary layer thickness; c_m = concentration of component at the membrane surface, c_P = concentration in the permeate and c_b = concentration in the bulk of the feed.

D/L is replaced by the mass transfer coefficient k:

$$J = k\ \ln\left[(c_m - c_p)/(c_b - c_P)\right] \tag{4.14}$$

This is the general equation for any component. For a component which is completely rejected by the membrane ($c_P = 0$), this equation becomes

$$J = k\ \ln\left[c_m/c_b\right] \tag{4.15}$$

This is the more familiar representation of this equation. The term c_m/c_b is known as the membrane polarisation ratio. Several investigators have suggested that the concentration at the surface eventually reaches a value at which a gel is formed, i.e. ($c_m = c_g$), where c_g is the gel concentration. Thereafter no further increase in concentration occurs and the flux remains constant, thus providing a (further) explanation for the existence of a pressure-independent region. The equation also predicts a straight line relationship between the flux and the log of the concentration, which has been found by many investigators. By extrapolating the experimental data to zero flux the gel concentration for various feedstocks and proteins has been estimated. Some values cited by Cheryan (1989) are given in Table 4.5. However, there have been some discrepancies between

Table 4.5. Gel concentrations for different proteins

Feed	Gel concentration c_g
Skim milk	20–25% protein
Full cream milk (3.5% fat)	9–11% protein
Acid whey	30% protein
Sweet whey	20–28% protein
Gelatin	22–30% protein
Egg white	40% protein
Defatted soy extract	20–25% protein

Taken from Cheryan (1989).

these values and those determined for the proteins by more conventional methods, throwing some doubt on the validity of this type of analysis (Aimar, 1987).

A further explanation for the discrepancies could be that the rejection of the components is not 1.0, making eq. (4.15) not directly applicable.

Nevertheless these equations help explain in qualitative terms the observations that in the pressure-independent region, the flux can be increased by increasing the mass transfer coefficient. Because of this, considerable attention has been paid to the determination of this mass transfer coefficient.

There are various qualitative (empirical) relationships in the literature, which correlate mass transfer coefficient to physical properties, flow channel dimensions and operating parameters. Cheryan (1989) concluded that many of these are not very satisfactory. Dimensional analysis has also been frequently used:

$$Sh = A\ Re^a\ Sc^b$$

where Sh is the Sherwood number = (kd/D), Re is the Reynolds number = (vdp/μ) and Sc is the Schmidt number = (μ/pD), where d is the tube diameter and A, a and b are constants. For other flow situations the hydraulic mean diameter can be used, which equals 4 (cross-sectional area/wetted perimeter).

A much used form of the equation for turbulent flow is

$$Sh = 0.023\ Re^{0.8}\ Sc^{0.33} \tag{4.16}$$

Cheryan (1986) summarises some of the constants for different flow geometries and feed materials. Under turbulent flow conditions, the constant a ranged between 0.5 and 1.1, with 0.8 being a typical value. The value was 0.3 for laminar flow. Again dimensional analysis predicts that the flux rate can be increased by increasing the Reynolds number and by inducing turbulence. However, it has been noted that high flux rates have been observed at high shear rates under streamline flow conditions. More recently, Colman and Mitchell (1991) have described how pulsed flow and baffles have increased flux rates by a factor of 3, at a Reynolds number of 100. This was stated as giving a mass transfer value equivalent to a steady Reynolds number of 10 000 in unbaffled channels.

Although dimensional analysis predicts the importance of turbulence, the values predicted using these models are often lower than those measured in practice. Measurement often requires an estimate to be made for c_m, the concentration at the membrane surface. The reasons for these higher experimental values are attributed to factors other than diffusion, causing the transport of rejected materials back into the bulk of the solution. One such explanation for colloidal particles is the 'tubular pinch effect', whereby colloidal molecules were observed to move away from the tube wall.

Aimar (1987) describes the ultrafiltration of pseudoplastic fluids and reported that in the pressure-independent region, the value of the limiting flux J_L was found to be given by

$$J_L = Au^a c^b \tag{4.17}$$

which fits in well with conventional mass transfer theory. In short, the information derived from modelling gives qualitative data on the ways of improving mass transfer.

Some of the drawbacks of modelling for real systems stem from the complexity of real feeds. Most are multicomponent, with many compounds which are totally or partially rejected, with interactions between components. Their rheological behaviour is complex, and there are difficulties of measuring the physical properties of the solutions under conditions found within the membranes and a lack of accurate diffusion data for macromolecules.

There is a further suggestion that osmotic effects are likely to be more important than initially thought for ultrafiltration, because the osmotic pressure difference over the membrane depends upon the concentration at the membrane surface and not on that in the bulk solution. However, those components which contribute most to the osmotic pressure have a low rejection and therefore show little accumulation at the membrane surface. When the membrane system is operating at very low transmembrane pressures, say about 1 atmosphere, the osmotic pressure difference may be significant.

4.3.2 Fouling

In most practical applications, fouling of the membrane takes place and this is a major operating problem in ultrafiltration. Fouling material collects on the surface of the membrane (and perhaps internally) and gives rise to a steady decline in the permeate flux (see Fig. 4.5). This could be particularly important for continuous processes operating at steady state, where a long-term decline in the flux would be extremely detrimental to the process. It could also give rise to a reduced life for the membrane, due to more stringent cleaning regimes being needed to remove the fouling.

Fouling is almost impossible to avoid. Removal of colloidal and particulate matter is of paramount importance prior to processing and should always be carried out. However, both Fane and Fell (1987) and McGregor (1986) have described situations where fouling was apparent, even with 'pure' water. McGregor also reported that prolonged exposure to 200 ppm of sodium hypochlorite caused considerable flux decline. When more complex materials are involved, such as proteins, interactions can occur between the proteins and the membrane material; for example proteins may bind to the membrane by hydrophobic effects, charge transfer such as hydrogen bonding and electrostatic interactions, or through combinations of these. Conditions that minimise the amount of binding to the

membrane should be useful in reducing fouling. The two characteristics which appear to strongly influence fouling are the physicochemical properties of the membrane and porosity and morphology of the surface. It is also not easy to assess the individual contributions made by concentration polarisation and fouling toward flux decline. Wu *et al.* (1991) suggest that concentration polarisation is responsible for the rapid initial flux decline, which is followed after about ten minutes by a long and gradual flux decline, caused by fouling. Fane and Fell (1987) report that the flux decline due to concentration polarisation is largely reversible and is therefore different in nature to that caused by fouling. Work on measuring the membrane resistance (R_m) during these early stages of concentration polarisation showed an increase in its value, suggesting that some fouling was taking place within the pores, as well as on the surface. Other suggestions are that there are three phases in flux decline: (1) due to concentration polarisation, taking place very quickly (seconds); (2) due to initial adsorption of protein onto the surface; and (3) due to further adsorption and an increase in deposit thickness.

There have been relatively few attempts to present a mechanistic model for long-term flux decline, because the issues involved are extremely complex. It has been pointed out by Wu *et al.* (1991) that the adsorption of protein on the membrane alone can change the filtration characteristics, and that the amount and rate of protein adsorption vary with many factors, including the nature of the membrane, type of protein, its concentration, pH and ionic strength of the protein. Any attempts to model the process taking place needs much experimental data and the parameters depend on the conditions used. One such attempt has been described by Suki *et al.* (1986), which assumes that protein aggregation in the concentrated layer next to the membrane is based on flocculation theory. Using data available for BSA, the flux is calculated as the aggregate layers build up. Qualitatively, the model predicts increased flux decline for higher feed concentrations. However, it does not allow for differences in the membrane properties.

Owing to these difficulties, there are also a number of models (empirical, phenomenological) to describe the fouling process. Unfortunately they do not offer an explanation for the reasons why fouling occurs, although they are useful for predicting flux decline with time. Some of these have been reviewed by Cheryan (1986), Fane and Fell (1987) and Wu *et al.* (1991).

The simplest is based on an exponential relationship between flux and time:

$$J = J_0 e^{-bt} \tag{4.18}$$

where J = flux at time (t) and J_0 = flux at time zero and t is time or

$$J = J_0 V^{-b} \tag{4.19}$$

where V = volume permeated and b is an index of fouling.

One drawback of these equations is that they predict that the flux will eventually decline to zero, which is not the case in practice. Cheryan (1986) has listed seven models based on this exponential decay. Other types of model use the resistance in series approach, where flux decline results from an ageing process in the deposit. Some of these are reviewed by Fane and Fell (1987). More recently, Wu *et al.* (1991) have developed a semi-empirical model, which is based firstly on postulating that the rate of flux change is

directly proportional to the flux itself, and secondly using an exponential attenuation term to describe the effect of membrane fouling on the rate of flux decline. The main parameters in the model are a rate constant related to concentration polarisation (k_P) and a rate constant related to membrane fouling, which can easily be determined, due to a linear relationship between $\ln(\ln(J/J_i))$ and time where J_i is the final (steady) flux. Data is presented for a whey protein isolate and the model is also used to estimate these parameters for some other fouling systems.

Considerable advances have been made in understanding the factors which affect flux rates and fouling for dairy products, particularly with whey processing.

This is considered in more detail as it provides a good illustration of the complex interactions of solutes during an ultrafiltration process. The compositions of sweet and acid wheys are given in Table 4.6. More details are provided by Hayes *et al.* (1974), Lee and Merson (1976), Glover (1985), and Heng and Glatz (1991). Fouling characteristics were different in sweet whey and acid whey. Initial fluxes are slightly higher for sweet whey than acid whey, which was attributable to its having only about half the amount of calcium. However, fouling has been reported to be just as severe in sweet whey as in acid whey, attributed mainly to calcium phosphate. For sweet whey, the flux can be improved by the following procedures: heating at 60°C for 30 min prior to ultrafiltration, thereby precipitating the calcium phosphate; demineralisation by ion exchange or electrodialysis (see Chapter 6), the reduction in ionic strength reducing protein aggregation; using calcium sequestering agents, such as EDTA and sodium hexametaphosphate; and lowering the pH to about 3, which increases the solubility of calcium phosphate. An alternative procedure is to demineralise the whey, adjust the pH to 4.6 and allow the deposit to settle out.

For acid whey, pasteurisation and adjustment of the pH to between 5.2 and 5.9 brings about an improvement in flux, and this is more marked for hydrochloric acid whey compared to sulphuric acid or lactic acid wheys (Hayes *et al.* 1974). Kuo and Cheryan (1983) found that fouling could be minimised by pH adjustment to between 2 and 3, followed by removal of resulting insoluble particulates. At low transmembrane pressures it was observed that higher flow rates had a beneficial effect on fouling, whereas at higher transmembrane pressures, higher flow rates significantly increased the fouling rate. They suggested that fouling takes place initially on the surface, which is followed by

Table 4.6. Composition of sweet whey and acid whey and skim milk (% w/v)

	Sweet whey	Acid whey	Skim milk
Lactose	4.9	5.1	4.8
Protein	0.5	0.6	3.3
NPN	0.04	0.04	0.04
Fat	0.3	0.1	0.15
Ash	0.6	0.7	0.7
Lactic acid	0.2	tr	—
pH	5.7–6.4	4.1–4.4	6.6–6.7

fouling within the pores. Patocka and Jelen (1987) found that all treatments resulting in the elimination of free calcium improved flux. Heng and Glatz (1991) determined that the extent of flux decline increased with the level of calcium and phosphorus deposition during processing. Electron microscopy has been used to observe the fouling deposits. Lee and Merson (1976) showed the complexity of fouling; several types of morphology depending upon the treatments and conditions used. Glover (1985) reported that deposits 1 μm in thickness have been observed, with some of the particulate matter penetrating up to 2 μm into the membrane (Nisbett *et al.*, 1981). Of the proteins present, fouling is mainly attributable to β lactoglobulin, which forms sheets over the membrane surface. More advanced analytical techniques now available, such as IR and X-ray photoelectron spectroscopy can be used to identify fouling compounds and residues after different cleaning regimes (Daufin *et al.*, 1992).

Some recent work with permeates from different types of milk and whey has shown that some of the low molecular weight components in milk, such as calcium and lactose, also cause fouling to take place (Ramachandra Rao *et al.*, 1994). This is contrary to the evidence of Kessler *et al.* (1982).

Fouling and concentration polarisation behaviour have been compared by McGregor (1986) for a wide range of commercially available membranes, using a number of complex protein extracts. He defines and uses a polarisation/fouling index and a fouling index to compare their performance.

To summarise, during an ultrafiltration process the flux declines. There is usually a rapid decline when the process fluid replaces the water due to concentration polarisation. Further decline will result from the increase in the bulk viscosity of the feed and the onset of fouling. When the flux reaches a predetermined low value, the membranes will need cleaning in order to restore the flux.

Methods and strategies for reducing fouling are discussed by Fane and Fell (1987).

4.3.3 Factors affecting flux

Energy input

The main source of energy is the pumping energy. The power utilisation (W) is related to the pressure (head) developed and the mass flow rate as follows:

$$W = m'hg \tag{4.20}$$

where m' = mass flow rate (kg s^{-1}), h = head developed (m) and g = acceleration due to gravity (9.81 m s^{-2}).

This energy is largely dissipated within the fluid as heat and will result in a temperature rise. Cooling may be required to prevent this. Factors that improve flux rates usually increase energy consumption.

There has been a great deal of experimental investigation into the factors affecting the permeate flux. For a simple system, the average pressure of the product is $(P_1 + P_2)/2$, where P_1 and P_2 are the inlet and outlet pressures respectively.

In most cases the pressure on the permeate side of the membrane is atmospheric. Therefore, the average pressure driving force over the membrane is given by $(P_1 + P_2)/2$;

this is often referred to as the transmembrane pressure. Pressure may also be applied to the permeate side, in order to reduce the initial flux and concentration polarisation during the transition from water to product. The pressure difference $(P_1 - P_2)$ will depend upon the flow rate and viscosity of the fluid.

The processing variables which have been found to be important are as follows:

(1) *Pressure.* An increase in the pressure increases the permeate flux, usually up to a limiting value, above which the permeate rate becomes independent of pressure: for these reasons we talk of a pressure-dependent and pressure-independent region. The pressure-independent region has been linked with the formation of a gel-permeation layer, adjacent to the membrane. Higher pressures are said to cause compaction of the gelled layer near the membrane surface and perhaps the membrane itself.

(2) *Flow rate.* Increasing the flow rate increases turbulence and decreases the boundary layer thickness; this increases the permeate rate; the relationships between permeate rate, pressure and flow rate are shown in Fig. 4.6. An increase in the flow rate leads to an increase in the pressure, at which the onset of pressure-independence begins.

Higher flow rates give rise to higher frictional losses and higher energy requirements:

- for streamline flow conditions: pressure drop $\propto$ (flow rate)$^{1.0}$
- for turbulent flow conditions: pressure drop $\propto$ (flow rate)$^{1.8}$.

(3) *Temperature.* All investigators have found that increasing the temperature increases the permeate flux; the relationship between permeate flow rate and temperature is almost linear over the relatively narrow range of temperatures investigated. An increase of about 2% per °C rise in temperature is quite normal. Suggestions for the increase in permeate rate are: a change of porosity of the membrane, decrease in viscosity of the feed, higher diffusion rates or increased solubility of the diffusing material in the membrane.

In practice, for foodstuffs, chemical and microbiological considerations have a big influence on the operating temperatures used. Temperatures between 5 and 45°C are often avoided, as microorganisms may grow. With non-cellulosic membranes, the highest temperatures which cause no loss of activity or functionality are used; often in the temperature range 50 to 60°C. Higher temperatures (70–90°C) are used for cleaning and disinfecting the equipment.

As well as the operating conditions, the nature and composition of the feed affect the permeate rate; particularly its chemical nature and its viscosity. More detail will be provided in the applications section. There is some interdependence; for example, temperature affects viscosity and all the latter three factors influence the degree of turbulence.

(4) *Turbulence.* The role of turbulence has been described in an earlier section. The extent of turbulence is also influenced by temperature and composition and is characterised by the Reynolds number (eq. (4.16)).

The two extremes are laminar flow conditions, usually combined with high shear rates, through to fully developed turbulent flow.

Other factors such as pH and osmotic pressure may also influence the permeate flux, as well as membrane configuration (see Chapter 3).

4.4 DIAFILTRATION

Introduction

An extension of ultrafiltration employs the addition of water at some stage during the concentration process. It should be remembered that during ultrafiltration, the concentration of any component in the retentate can never decrease (unless there is a true negative rejection). At best, for zero rejection, it will remain constant. However the amount (yield) of a component of low rejection value is significantly reduced, as is the dry weight composition, compared to a substance with a much higher rejection.

To effect a reduction in concentration, the retentate needs to be diluted with water; such a process is known as diafiltration. The net effect of diafiltration is to wash out more of the lower molecular weight components. The two main modes of operation are *discontinuous diafiltration* (DDF) and *continuous diafiltration* (CDF).

DDF is where the water is added back to the retentate intermittently. For example a dilute extract may be concentrated by a factor of 10, by normal ultrafiltration. Water could then be added back to the extract, say to restore the original volume. The diluted retentate could then be further processed (diafiltered) to further reduce the volume of the retentate. Further water can be added as required. Extensive diafiltration should eventually reduce the concentration of low molecular weight compounds to very low levels. DDF can be analysed in the same way as a batch ultrafiltration process, with account being taken of dilution effects. Lewis and Finnigan (1989) found DDF useful for removing glucosinolates from rapeseed meal extracts. After a fivefold concentration, followed by dilution back to the starting volume and a further fivefold concentration, the protein content had increased from 45.3 to 83.0% (dwb) and the glucosinolate concentration was reduced from 2.5% to 0.3%. However glucosinolate rejection values were greater than zero and there were considerable losses of protein in the permeate.

There are many similar examples of diafiltration processes in the literature.

4.4.1 Washing out at constant volume

CDF involves the continuous addition of water, at a rate equal to the rate at which permeate is removed. Normally this would be termed washing out. This is probably the most common form of diafiltration and can be subjected to mathematical analysis (see Fig. 4.8).

Let

system volume = V_0

permeate flow rate = P

water flow rate = W

At steady state $W = P$

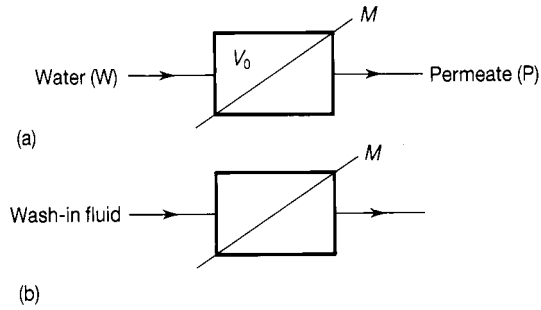

Fig. 4.8. Diafiltration: (a) washing out; (b) washing in.

Solute balance:

$$V_0 c = V_0 (c + \mathrm{d}c) + P c_P \, \mathrm{d}t$$

$$-V_0 \, \mathrm{d}c = P c_P \, \mathrm{d}t$$

For $R = 0$, $c = c_P$

$$-V_0 \, \mathrm{d}c = P c \, \mathrm{d}t$$

$$-V_0 \int_{c_0}^{c} \frac{\mathrm{d}c}{c} = P \, \mathrm{d}t$$

$$-V_0 \ln\left(\frac{c}{c_0}\right) = Pt$$

$$\ln\left(\frac{c_0}{c}\right) = \frac{Pt}{V_0} = \frac{V}{V_0} \tag{4.21}$$

where V = cumulative permeate volume.

The ratio V/V_0 is termed the number of diavolumes removed. Equation (4.21) gives the number of diavolumes required to bring about a certain removal of the freely permeating species ($R = 0$). Thus, a tenfold reduction in concentration would require the removal of 2.303 diavolumes.

When $R \neq 0$; $c_P = c\,(1 - R)$

$$\frac{-V_0 \, \mathrm{d}c}{c(1-R)} = P \, \mathrm{d}t$$

$$\frac{-V_0}{(1-R)} \int_{c_0}^{c} \frac{\mathrm{d}c}{c} = \int P \, \mathrm{d}t$$

$$\frac{V_0}{(1-R)} \ln\left(\frac{c_0}{c}\right) = Pt$$

Therefore

$$\ln\left(\frac{c_0}{c}\right) = (1-R)\left(\frac{V}{V_0}\right) \tag{4.22}$$

Again, this equation is only applicable if R remains constant.

Thus a component with a rejection of 0.1 would require the removal of 2.59 diavolumes to reduce its concentration ten times. (cf. 2.3 diavolumes for $R = 0$.) Glover (1985) stated that in milk, the most efficient diafiltration was to concentrate to about 2, followed by continuous diafiltration.

If the analysis is done on the permeate, the equation becomes

$$\ln\left(\frac{c_0(1-R)}{c_P}\right) = (1-R)\left(\frac{V}{V_0}\right) \tag{4.23}$$

This shows how the permeate concentration changes with the number of diavolumes, or with time.

All these analyses have ignored the void volume V', which can be accounted for by an additional term in eq. (4.21), as follows:

$$\ln(c/c_0) = V/V_0 - V'/V_0$$

Analysis shows that the optimum concentration for the retained species ($R = 1$), to minimise the processing time, is given by c_g/e, where c_g is the gel layer concentration (see Table 4.5). Further analyses for optimising the processing time for diafiltration of soya bean extracts and cheese whey are performed by Ali Asbi and Cheryan (1992). The optimum appeared to be a combined process where the feed is first concentrated by ultrafiltration, followed by continuous diafiltration.

Washing-in

Washing-in applications can be analysed in a similar way:

$$\text{for } R = 0 \qquad \ln\left(\frac{c_f}{c_f - c}\right) = \frac{V}{V_0} \tag{4.24}$$

$$\text{for } R \neq 0 \qquad \ln\left(\frac{c_f}{c_f - c}\right) = \left(\frac{V}{V_0}\right)(1-R) \tag{4.25}$$

where c_f = concentration of component in the wash-in fluid.

Washing-in procedures have been used in binding studies, for example protein solution is placed in the cell and the binding component is added in the wash-in solution. Analysis of the component in the permeate together with a knowledge of its rejection characteristics and a mass balance will permit the total quantity of the component bound to the protein to be calculated. Further examples and details are provided by Cheryan (1986).

4.4.2 Diafiltration applications

Diafiltration is widely employed to remove low molecular weight components, whether they be anions, cations, sugars, alcohol or antinutritional compounds. It therefore offers an alternative process to ion exchange, electrodialysis and dialysis. One interesting medical application involves haemodialysis, for kidney patients.

Another interesting application involves the use of membranes to process alcoholic drinks. The membranes allow alcohol and water to permeate but retain other components within the drink. If water is added back to the concentrate, the net result is that alcohol is reduced. Here also is an example where there is an interest in the permeate.

4.4.3 Protein fractionation

There is potential for using ultrafiltration, combined with diafiltration, to separate individual proteins from a mixture to produce reasonably pure fractions, provided that the molecular weights of the proteins are well distributed and the membranes have a narrow pore size distribution. Lewis (1982) illustrates a scheme for separating three proteins of molecular weight 5000 (A), 50 000 (B) and 500 000 (C), present in equal concentrations. Fractionation is achieved in a two-stage process, using membranes *X* and *Y*, with molecular weight cut-off (MWCO) values of 10 000 and 100 000 respectively, in the first and second stages (see Fig. 4.9). Two situations are illustrated, using a membrane with ideal rejection values and those with rejection values found in practice (real membrane). Rejection values used for these calculations are given in Table 4.7.

The feed is concentrated in the first stage by a factor of 10 and the permeate (P_1) consists mainly of A. The concentrate is diluted back to its original volume with water and ultrafiltered by a factor of 10, using membrane *Y*. The concentrate (C_2) and permeate (P_2) from the second ultrafiltration process comprise predominantly C and B respectively. The compositions of the three streams (dry weight basis) are given in Table 4.8, for the ideal membrane and the real membrane; eqs. (4.6) and (4.8) can be used to perform such calculations.

Better separations would also be achieved by using higher concentration factors.

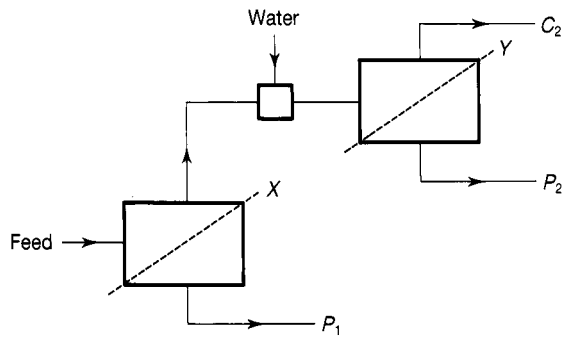

Fig. 4.9. Fractionation of proteins A, B and C, with membranes *X* and *Y*; P_1, permeate from first stage; P_2, permeate from second stage; C_2, concentrate from second stage.

Table 4.7. Membrane data for protein fractionation

Membrane	MWCO	Rejection (ideal)			Rejection (real)		
		A	B	C	A	B	C
X	10 000	0	1	1	0.1	0.99	1.00
Y	100 000	0	0	1	0.05	0.10	0.99

Table 4.8. Composition of different streams

	Composition (dry weight basis)					
	Ideal			Real		
	A	B	C	A	B	C
Feed	33.3	33.3	33.3	33.3	33.3	33.3
P_1	100	0	0	97.4	2.6	0
C_2	0.9	9.0	90.1	1.3	11.0	87.7
P_2	9.1	90.9	0	11.3	86.4	2.3

Further purification of each stream could be accomplished by more extensive diafiltration. It can be seen that although there is some scope for enrichment of proteins, true fractionation is difficult. The major limitation would appear to be due to the diffuse nature of the membrane and the wide distribution of pore sizes. Cheryan (1986), using a similar type of analysis, came up with a similar set of data and arrived at similar conclusions.

An alternative procedure would be to employ continuous diafiltration to wash out each of the proteins in turn, starting with the smallest.

McGregor (1986) has examined a wide range of membranes for separating complex mixtures of proteins. Some membranes showed reasonably sharp characteristics in terms of the permeate, i.e. it was possible to produce permeates with very little protein above a certain molecular weight, but with a mixture of proteins in the concentrate.

A novel approach to enrichment, in situations where the fraction of interest represents a small proportion of the total protein, has involved depositing inorganic or organic compounds within the matrix of a porous stainless steel tube. These components separate on the basis of charge or size exclusion. Immunoglobulin G has been enriched from 8% to 20% in cheese whey by this method (Thomas *et al.*, 1992).

4.5 ULTRAFILTRATION APPLICATIONS

Some applications of ultrafiltration will now be discussed, which take advantage of the opportunity to concentrate macromolecules or to remove small molecular weight components, at ambient temperature, without changing pH or ionic environment.

A wide range of membrane materials is available, the most common being cellulose acetate, polyamides, polysulphones and polyethersulphones; each with different flux characteristics, rejection values, and other physicochemical characteristics, such as charge and extent of hydrophobicity.

Molecular weight cut-offs range from 2000 to 300 000, with operating temperatures up to 80°C, over the pH range 1 to 14.

Ultrafiltration is also very useful for recovering valuable components from food-processing waste stream and fermentation broths. Probably the greatest impetus has come from the dairy industry and dairying applications. However, in all applications, flux decline due to concentration polarisation and fouling are probably the two most important practical aspects.

4.5.1 Dairy applications

Milk is chemically complex, containing components of a wide range of molecular weights, such as protein, fat, lactose, minerals and vitamins. It also contains micro-organisms, enzymes and perhaps antibiotics and other contaminants. An idea of the complexity of milk is given by Walstra and Jenness (1984), who list well over 50 components. Whole milk contains about 30–35% protein and about the same amount of fat (dry weight basis). Therefore it is an ideal fluid for membrane separation processes, in order to manipulate its composition, thereby providing a variety of products or improving the stability of a colloidal system. The same applies to skim-milk, standardised milk and some of its by-products such as cheese whey. Skim milk can be concentrated up to seven times and full-cream milk up to about five times (Kosikowski, 1986). Milk can be derived from a number of different species, cows' milk being the most common, with milk from buffalo, goats and sheep being drunk in substantial quantities throughout the world. Milk is either consumed in its liquid form or converted to a wide variety of products. Surplus milk is usually preserved as skim milk powder and butter or anhydrous butterfat. At one time the most valuable component was the fat, with cream products and butter fetching high returns. Skim milk was therefore a by-product of cream and butter manufacture, along with lesser quantities of buttermilk. Most of the skim milk was dried, and in some situations fed to animals. However, a further important trend over the last ten years has been the move to a more health-conscious diet, and in this sense skim milk is more widely used as the starting material for yoghurts, low-fat cheeses and other desserts. Ultrafiltered milk also forms the starting material for some of these types of product (de Boer and Koenraads, 1991).

Cheese is a very important product derived from milk. In cheese production, most of the fat and the casein fractions are incorporated into the curd. However the by-product of cheese manufacture is whey, which incorporates the whey proteins (about 20% of milk protein). The compositions of whey and skim milk are given in Table 4.6.

An IDF publication (International Dairy Federation, 1979) gives a summary of the rejection values obtained during the ultrafiltration of sweet whey, acid whey, skim milk and whole milk, using a series of industrial membranes. These results are summarised in Table 4.1. It should be noted that protein rejections are based on Kjeldahl nitrogen × 6.38. Where this is the case, it is also measuring non-protein nitrogen (NPN). Rejection values obtained may not be a true reflection of the behaviour of the protein. Such

rejection values could be lower than expected for materials containing substantial amounts of NPN. One example is chhana whey, which is produced from a heat-coagulated cheese and contains substantial NPN (Jindal and Grandison, 1992).

However, low rejection values could also be indicative of excessive protein leakage, and may well warrant investigation if not expected. Rejection values based on true protein can be determined by Kjeldahl or by using electrophoresis or high performance liquid chromatography (HPLC) for analysis and will give a clearer picture of the behaviour of the proteins.

Rejection values for ash are interesting and are higher than would be expected from their molecular weight. This would suggest that binding of minerals to the protein is occurring. During ultrafiltration of whey and buttermilk, Hiddink *et al.* (1978) observed that at pH 6.6, anions such as Cl^- and NO_3^- were preferentially removed. However, at pH 3.2, cations such as Na^+, K^+, and Ca^{2+} were preferentially removed. This is an example of the Donnan effect. Maximum ash removal was obtained by ultrafiltration at pH 6.6, followed by diafiltration at pH 3 to 3.5.

Bastian *et al.* (1991) compared the rejection values during ultrafiltration and diafiltration of whole milk. They found that the rejection of lactose, riboflavin, calcium, sodium and phosphorus was higher during diafiltration than ultrafiltration. Diafiltration of acidified milk gave rise to lower rejections of calcium, phosphorus and sodium. Premaratne and Cousin (1991) have performed a detailed study on the rejection of vitamins and minerals during ultrafiltration of skimmed milk. During a five-fold concentration the following minerals were concentrated by the following factors: Zn (4.9), Fe (4.9), Cu (4.7), Ca (4.3), Mg (4.0) and Mn (3.0), indicating high rejection values. On the other hand, most of the B vitamins examined were almost freely permeating.

The use of nanofiltration or partial demineralisation has been discussed by Kelly *et al.* (1991) and its effects on lactose crystallisation, which was improved by about 8% at a concentration factor of 3, by Guu and Zall (1992).

Both cheese whey and skim milk contain substantial protein and other nutrients. A great deal of attention has been paid to ultrafiltration of these products to increase their functionality and hence profitability.

Cheese whey contains only about 10–12% protein on a dry weight basis. However the proteins are soluble and have excellent functional properties. The main thrust has been toward using ultrafiltration to increase the protein content, in order to produce concentrates, which could then be dried to produce high protein powders (concentrates and isolates) with useful functional properties. Some typical concentration factors (f) used are as follows:

$f = 5$	protein content (dwb) about 35% (similar to skimmed milk)
$f = 20$	protein content about 65%
$f = 20$; plus diafiltration	protein content about 80%

The product starts to become very viscous at a concentration factor of about 20. Therefore if a protein concentrate with a higher total solids is required, diafiltration is required.

However, some of the minerals are difficult to remove. In order to produce protein isolates, containing over 90% protein, ion exchange is often used. Whey from some cheese varieties, e.g. chhana, may contain substantially less true protein; because of this, concentration factors up to 40 are possible (Jindal and Grandison, 1992).

The most effective concentration is achieved when the rejection for protein is 1.0 and that for lactose and minerals is 0. Figure 4.10 shows how the protein content of the whey concentrate, measured on a dry weight basis, is likely to change with concentration factor for this ideal situation and also for a practical situation, where protein, lactose and mineral rejections would be about 0.98 and 0.1 and 0.2 respectively. For the practical situation, the protein content is always lower, at any concentration factor. There are also slight losses of protein in the permeate.

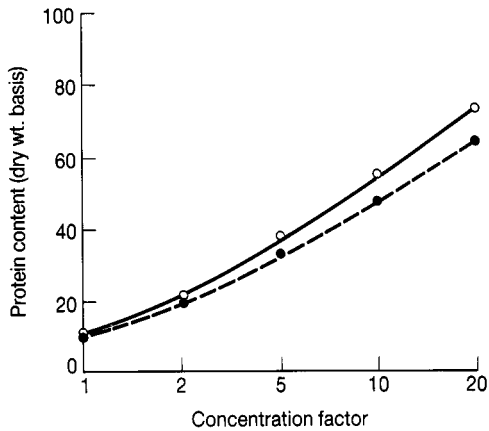

Fig. 4.10. Illustration of change of protein content (dry weight basis) during ultrafiltration of whey; o ideal situation; • practical situation.

More recently inorganic membranes have been used for whey protein concentration and a study of the fouling of these membranes has been made by Daufin *et al.* (1991). Calcium phosphate was found to be partly responsible for flux decline and the role of protein increased as its concentration increased and its pH fell. Coolbear *et al.* (1992) have examined the effects of some thermophilic proteinases for cleaning ultrafiltration membranes fouled during whey processing. Performance was synergistically enhanced using the anionic detergent SDS in combination with the enzyme. Bohner and Bradley (1992) have described an effective cleaning and disinfecting procedure for polysulphone membranes, fouled by whey solids.

The permeate from ultrafiltration of whey contains about 5% total solids, the predominant component being lactose. Since this is produced in substantial quantities, the economics of the process are dependent upon its utilisation. It can be concentrated by reverse osmosis and hydrolysed to glucose and galactose to produce sweeteners or fermented to produce alcohol or microbial protein.

Skim milk has been investigated also. However because of its original higher protein content, concentration factors of about 7 are the maximum achievable. Protein concentrates based on skim milk have not received the same amount of commercial interest as those based on whey proteins. However, it has been suggested that the concentrates can be further modified to produce an interesting range of products with good whipping and foaming characteristics. Rajagopalan and Cheryan (1991) reported that it was not possible to produce a pure protein isolate by ultrafiltration and diafiltration of skim milk, due to a high mineral rejection. An isolate containing about 90% protein and 8% ash was obtained.

Yoghurt and other fermented products have been made from skim milk and whole milk concentrated by ultrafiltration (Renner and El-Salam, 1991). Whey protein concentrates have also been incorporated (de Boer and Koenraads, 1991). Production of labneh, which is a strained or concentrated yoghurt at about 21% total solids, has been described by Tamime *et al.* (1991), by preconcentrating milk to 21% TS. Inorganic membranes have also been used for skim milk, and Daufin *et al.* (1992) have investigated the cleanability of these membranes using different detergents and sequestering agents.

As well as exploiting the functional properties of whey proteins, full cream milk has been concentrated by UF, prior to cheesemaking. The UF concentrate has been incorporated directly into the cheese vats. Some advantages of this process include: increased yield, particularly of whey protein, lower rennet and starter utilisation, smaller vats, or even complete elimination of vats, little or no whey drainage and better control of cheese weights. Lawrence (International Dairy Federation, 1989), suggests that concentration below a factor of 2 gives protein standardisation, reduced rennet and vat space, but no increased yield. At concentration factors greater than 2, an increased yield is found.

Some problems result from considerable differences in the way the cheese matures and hence its final texture and flavour. The types of cheese that can be made in this way include: Camembert type cheese, mozzarella, feta and many soft cheeses. Those which are difficult include the hard cheeses such as Cheddar and also cottage cheese; the problems are mainly concerned with poor texture. Some debate about compositional standards for cheeses produced by such technology exists. More discussion is given by Kosikowski (1986).

Further reviews on the technological problems arising during the conversion of retentate into cheeses are discussed by Lelievre and Lawrence (1988). Spangler *et al.* (1990a) have investigated how the quality of Gouda cheese was affected by concentration factor (3.6–5), rennet concentration and coagulation temperature. The best cheeses were made from the five-fold concentration and a rennet concentration of 0.14%. Higher rennet concentrations lead to the development of very bitter flavoured cheese. Spangler *et al.* (1990b), for example, found that Gouda production from ultrafiltered milk could be improved by attention to detail; such as preacidification of milk prior to ultrafiltration; the amount of rennet was also very important. Sachdeva and Reuter (1991) produced acceptable chhana by ultrafiltration; it had a lower moisture content, giving a yield improvement of 31.4% (product basis) and 16.4% on a dry matter basis. Quarg is also produced from ultrafiltered milk.

Whole milk has been concentrated five times by ultrafiltration and the retentate heated at 120°C for 5 min before being recombined with the permeate prior to pasteurisation (Kosikowski and Mistry, 1990). This procedure is claimed to produce an extended shelf-life product with a superior flavour to a conventionally pasteurised product.

Sweetsuir and Muir (1985) have investigated the production of sterile concentrates, produced by the ultrafiltration of skim milk, with and without the addition of fat. Such concentrates were able to withstand sterilisation at 120°C for 7 min. The organoleptic qualities were improved by the presence of fat and the heat stability was improved by procedures which reduced the levels of salts in the concentrates.

Protein hydrolysates produced from milk proteins have been discussed by Donnelly (1991).

Ultrafiltration is an extremely valuable method of concentrating and recovering many of the minor components, particularly enzymes from raw milk, many of which would be inactivated by pasteurisation. Such enzymes are discussed in more detail by Kosikowski (1988). On-farm ultrafiltration processing of milk has been suggested as a way of reducing transportation costs, but is only viable for long distances, i.e. greater than 100 km, and is not likely in the UK; the permeate can be fed to pigs.

Further reviews on membrane processing of milk are provided by Glover (1985, 1986), Renner and El-Salam (1991), El-Gazzar and Marth (1991) and International Dairy Federation (1991).

4.5.2 Oilseed and vegetable proteins

Crops such as soyabean, cottonseed, sunflower and maize are grown primarily for oil. After its extraction a solid residue remains which is often rich in protein. However, certain toxic components may also be associated with the residues. There have been many laboratory investigations into the use of ultrafiltration for extracting proteins from these residues, or removing the toxic residues. Lewis (1982) and Cheryan (1986) have reviewed the more important of these. For soya, the separation of low molecular weight peptides from soy hydrolysates, with the aim of improving quality; the dissociation of phytate from protein, followed by its removal by ultrafiltration; the removal of oligosaccarides; the removal of trypsin inhibitor and comparisons of performance of different membrane configurations. For cottonseed, the use of different extraction conditions has been evaluated, as have the functional properties of the isolates produced by ultrafiltration. Investigations were performed with sunflower and alfalfa to remove the phenolic compounds responsible for the colour and bitter flavour.

Many have been very successful in their objectives and good quality protein concentrates and isolates have been produced, particularly with soyabean. For example, Nichols and Cheryan (1981) showed an 86% recovery of soya protein by a process involving multiple extraction, centrifugation, ultrafiltration and diafiltration. Diafiltration was necessary to produce an isolate, but there was still a relatively high amount of minerals, due to a higher than expected mineral rejection.

However, in comparison to dairy processing, few have come to commercial fruition, mainly because of the economics of the process, dictated by the relatively low value of the products and the fact that acceptable food products can be obtained by simple

technology, such as isoelectric precipitation. A further problem arises from the fact that the starting residue is in the solid form, thereby imparting an additional extraction procedure. Extraction conditions may need to be optimised, with respect to time, temperature, pH and antinutritional factors. Most vegetable proteins have their minimum solubility somewhere between pH 4 and 7. They are much more soluble at high and low pH values, so the use of second-generation membranes has helped enormously with processing such extracts and cleaning of such membranes. However, ultrafiltration at high pH and high temperatures may cause protein hydrolysis and excessive loss of nitrogen in the permeate. This has been observed by Lawhon *et al.* (1978). It is usually advisable to prefilter the extracts down to below 50 μm to avoid excessive flux decline due to fouling.

A further problem arises from the complexity of oilseed and vegetable proteins, compared to milk products, evidenced by electrophoretic measurement. It is likely that many of these proteins are near their solubility limits after extraction and further concentration will cause them to come out of solution and promote further fouling. Fouling and cleaning of membranes was found to be a serious problem during ultrafiltration of rapeseed meal extracts (Lewis and Finnigan, 1989). Again it was possible by extensive diafiltration to produce an extract with a high protein content and virtually no glucosinolate (dry weight basis). The rejection of glucosinolate was higher than expected, suggesting some form of binding with the proteins.

Work has also been performed on potatoes and potato-processing wastes as well as bean and pea extracts. Waste streams from the vegetable industry have received attention, in order to recover starch and protein, as well as reducing biological oxygen demand (BOD). Some examples are given by Walters and Elliott (1983) and Koseoglu *et al.* (1991).

Kosikowski (1986) has reviewed some of the work on clarification of alcoholic beverages and sugar juices from cane, beet and fruit rind.

Fruit juices have been clarified successfully by membrane techniques; these are covered in more detail in Chapter 5. Vegetable juice processing is also important and is covered in more detail in the section on reverse osmosis (Chapter 3).

Applications in cereal processing have been reviewed by Koseoglu (1991); these include the recovery of solids from thin stillage, protein from wet-milling processes and foam proteins from fermentation broths. A further important aspect is the use of enzyme reactors for the breakdown of starch to sugars. This is discussed in more detail later in this chapter under membrane reactors (Section 4.5.4). Oil-processing applications are very much in their infancy. Potential applications in vegetable oil production and refining are reviewed by Koseoglu (1991). One application is in the solvent-oil extraction process to recover the solvent from the oil, thereby reducing the amount of distillation required. This is possible using reverse osmosis and ultrafiltration membranes but the main problem is compatibility of the membrane with the hydrocarbon solvent, which can lead to collapse of the structure. For polysulphone membranes, this problem can be partially overcome by presoaking the membrane with solvents of decreasing polarity; 50/50 solution of isopropanol/water, followed by 50/50 solution of isopropanol/pentane, followed finally by pentane. Research and development has led to a new generation of membranes, which are more resistant to hydrocarbons, which not only remove solvent but also refine the oil by removing lecithin, free fatty acids, and the majority of the

coloured pigments. The membrane processing is claimed to eliminate degumming, alkali refining, water washing and drying.

4.5.3 Animal products

Slaughterhouse wastes contain substantial amounts of protein. Two important streams that could be concentrated by ultrafiltration are blood and waste water.

Blood contains about 17% protein. It can be easily separated by centrifugation into plasma (70%) and the heavier erythrocytes (red blood corpuscles or cells – 30%). Plasma contains about 7% protein, whereas the blood corpuscles contain between 28 and 38% protein. The proteins in plasma possess useful functional properties, particularly gelation, emulsification and foaming. They have been incorporated into meat products and have shown potential for bakery products, as replacer for egg white.

Whole blood, plasma and erythrocytes have all been subjected to ultrafiltration processes (Ockerman and Hansen, 1988; Cheryan, 1986). The process is concentration polarisation controlled and flux rates are low. High flow rate and low pressure regimes are best. Gel concentrations were approximately 45% for plasma protein and 35% for red blood cells. The fouling characteristics of different blood fractions have been investigated by Wong *et al.* (1984). Whole blood was found to be the worst foulant, when compared to lysed blood and blood plasma.

One significant problem with the erythrocyte fraction is the dark colour, due to haemoglobin, which affects its acceptability in most foods. Methods to reduce this include oxidation using hydrogen peroxide or separation of the haem group from the haemoglobin. However, this latter procedure reduces the stability of the protein. A third procedure involves digestion of the haemoglobin with proteolytic enzymes and the use of membranes to recover the breakdown products.

Blood and other parts of the animal are used as raw materials for a wide range of active components.

Waste water, like many other factory wastes, has a high BOD, as well as containing up to 10% of the incoming protein (Cheryan, 1986), and processes for removal of BOD and recovery of fat and protein are described by him.

Another important material is gelatin, which can be concentrated from very dilute solutions by ultrafiltration. As well as concentration, ash is removed which improves its gelling characteristics. This is one example where there have been some high negative rejections recorded for calcium, when ultrafiltered at low pH.

Eggs have also been processed by UF. Egg white contains 11–13% total solids (about 10% protein, 0.5% salts and 0.5% glucose). Large amounts of egg white are used in the baking industry. The glucose can cause problems during storage and causes excessive browning during baking. Whole egg contains about 25% solids and about 11% fat, whereas egg yolk contains about 50% solids. It is unusual to evaporate eggs prior to drying, because of the damage caused. Both RO and UF have been investigated as a means of concentrating egg prior to drying; UF also results in the partial removal of glucose; further removal can be achieved by diafiltration. Flux values during UF are much lower than for many other food materials, most probably due to the very high initial protein concentration; rates are also highly velocity dependent and temperature dependent (Cheryan, 1986). It is possible to remove any remaining glucose by fermentation, prior to

spray drying. Egg white can also be concentrated to about 20% total solids by RO; again, the remainder of the glucose can be removed by fermentation before being spray dried. There are various processes to remove proteins from eggs (lysosyme by ion exchange) after they have initially been concentrated by RO and UF. There is a great deal of interest in removing cholesterol from yolk, with strong marketing advantages for the resulting product. However, despite its relatively low molecular weight, this has not been achieved by UF.

Extracts from cod offal have been subjected to ultrafiltration processes and three protein fractions were produced by a combination of ultrafiltration and pH precipitation. The extracts were hydrolysed but it was not found possible to further fractionate the hydrolysates by ultrafiltration (Vega and Brennan, 1987).

4.5.4 Biotechnology applications

There is a range of applications within the field of biotechnology, concerned with the transformation of components by biocatalysts. These include microorganisms, enzymes and plant or animal cells. The range of substrates and products is vast and the desired product may be either the cells themselves or their metabolic by-products, which may be intracellular or extracellular. UF membranes have a role, both in the primary production process, for example as a reactor for fermentation processes or enzymatic reactions, or in downstream processing for recovering the products.

Membrane-based bioreactors

Membrane-based bioreactors appear to be a very promising application for the production of ethanol, lactic acid, acetone and butanol, starch hydrolysates and protein hydrolysates; further refinements include the introduction of electrodialysis and pervaporation, into selective processes. The two main types of membrane reactor are the enzyme reactor and the fermentation reactor.

Enzyme reactors

Many industrial processes involve the use of enzymes to break down molecules, for example the hydrolysis of starch or proteins, or of simpler molecules such as sucrose or lactose. Most such reactions involving enzymes are of a batch nature and the enzyme needs to be inactivated or is lost at the end of the process because it is not easy to separate it from the product. The processing costs of batch processes are also high. There is considerable interest in continuous processes, where the enzyme is either immobilised or retained within the reaction vessel and reutilised, whereas the products are removed and reutilised. Ultrathin membranes have been investigated for both these types of application, in the form of enzymatic reactors. Immobilisation of enzymes provides an alternative method to overcome some of the disadvantages of using free enzymes. One system which has been much investigated is the immobilisation of the enzyme by adsorption and entrapment onto the outside of tubular membranes. Hollow fibre systems have been found useful for this purpose, with the enzyme being immobilised on the shell side of the reactor (see Fig. 4.11(a)). This is useful in situations where the product and reactant are normally much smaller than the enzyme and both diffuse into the membrane from the feed. One example is the hydrolysis of lactose. These types of reactor are

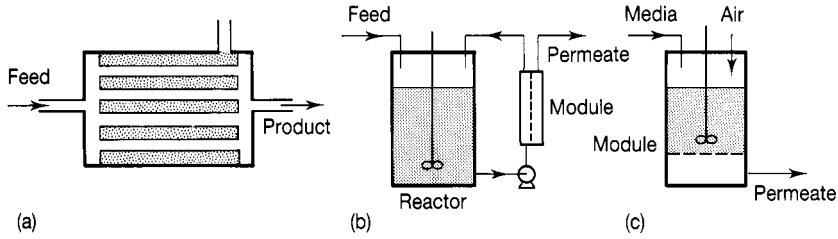

Fig. 4.11. Some immobilisation systems: (a) immobilisation within the shell space of a hollow fibre system; (b) continuous reactor linked to ultrafiltration module, for either enzymatic or fermentation reactions; (c) stirred cell or dead end reactor for enzymatic or fermentation reactions.

usually operated in plug flow mode. A variation on this theme is to immobilise the enzyme in the tubes and to pass the reactant through the shell; this is less common. The enzyme may also be immobilised within the membrane by incorporating it into the casting solution.

The second variant is where the enzyme is incorporated into the reaction vessel and the membrane not only physically retains the enzyme, but also allows a continuous removal of the product. This is not immobilisation in the strict sense of the word, but it does provide a mechanism for long-term usage. This arrangement is useful for hydrolysis of macromolecules, where the required end-product is often much smaller in molecular weight than the reactant or the enzyme. Dead-end or flow-through systems have been used (see Fig. 4.11(b, c)). The dead-end system uses the membrane unit both as the reaction unit and for separation and has been more widely investigated. Flow-through systems use a cross-flow filtration unit connected in series with the reaction vessel. This arrangement has two major advantages over the dead-end system, namely better induced turbulence to reduce concentration polarisation, which leads to poor activities in dead-end reactors after only a few hours' operation and a separate location for the reaction and separation vessels, thereby permitting each to be operated at its optimum set of working conditions, rather than at a compromise set of conditions.

Both systems operate essentially as a continuous stirred tank reactor (CSTR) and feed is supplied to replace the permeate removed. CSTRs are characterised by a wide distribution of residence times and are usually considered to be well mixed, i.e. the concentration of the feed to the membrane is the same as that in the reactor vessel. Thus at high conversions, the substrate concentration would be low; they are particularly useful for reactions which may be inhibited by substrate concentration; at first sight they may also appear useful because it helps reduce end-product inhibition. However, this is not necessarily the case because the end-product concentration in the permeate will be the same as that in the feed. A further feature for first- and second-order reactions is that a large volume is required for a CSTR compared to a plug flow reactor. The major advantages offered by this type of reactor are a high throughput and productivity, governed by a high enzyme concentration attainable and a constant and long-term use of the enzyme.

Cheryan (1986) has reviewed some of the potential applications for bioreactors for many types of protein and carbohydrate sources. Some other important aspects to be considered are the operating temperature and shear damage. Sometimes the optimum temperature for high activity may be one that also inactivates the enzyme quickly. Retained activity over a long period is an important function for membrane reactors.

Shear damage to enzymes was once thought to be important, although there is little experimental evidence to suggest that it is a major cause of reduced activity over the short term. Denis *et al.* (1990) examined the effects of pumping on the activity of pectate–lyase and found no loss of activity after 7 h pumping, but 36% loss of activity after 25 000 passes over a period of 6 days. Loss of activity due to adsorption could be reduced by selecting a membrane with the same charge as the enzyme, at the prevailing pH of the reaction. However, tissue culture and animal cells are much more sensitive to shear.

Vegetable proteins are more difficult to hydrolyse than animal proteins. The higher the levels of non-hydrolysable protein, the shorter will be the operational period for the reactor, due to the build-up of protein within the system. Cheryan and Deeslie (1983) reported run lengths of up to 90 h, during the hydrolysis of soya protein at 50°C, with yields between 85 and 94% and productivities (wt product/wt enzyme used) about 7 times higher than the equivalent batch process. There is scope for optimising the performance of such a reactor, in terms of temperature, enzyme concentration, substrate concentration, reactor volume and flow rate (also equivalent to permeate rate).

Protein hydrolysates have many uses, such as flavour enhancers, improvement of functional properties, and in special dietary or medical applications, some of which have been recently reviewed by Donnelly (1991).

Starch hydrolysis for the production of glucose syrups has also received some attention. There are two stages to the process, namely liquefaction and saccharification. Ideally, it would be desirable to perform both stages simultaneously. Liquefaction is difficult to achieve with enzymes at appreciable starch concentrations, because of the high viscosities involved (de Silva, 1991), although maltose has been produced by starch in a direct process (Nakajima *et al.*, 1990). Saccharification, which makes use of glucoamylase enzymes, is more suited to an enzyme membrane process as the batch process can take up to 48 h and the substrate is less susceptible to concentration polarisation and fouling, due to its lower viscosity.

Darnoko *et al.* (1989) examined the saccharification of cassava starch by glucoamylase in a dead-end stirred cell reactor. Essentially pure glucose was found in the permeate solids. Starch conversions were 64% at 25°C and 97% at 55°C. Productivity was 10–11 times better than a batch reactor over a 24 h period.

de Silva (1991) has studied the saccharification of starch in a CSTR-recycle membrane reactor, using maltodextrins (DE 17) as the feed material, at concentrations between 10 and 30% total solids. A tubular membrane was used with a molecular weight cut-off of 4000. The performance of the reactor was evaluated for different feed concentrations (10–30% TS), different enzyme concentrations and temperatures between 45 and 56°C. At 56°C and a feed concentration of 10% TS, a product containing 95% glucose was obtained, measured after 6 h operation. A mathematical model was developed to describe the process. This included the following terms: reaction kinetic parameters for

the enzyme, including product inhibition, and temperature effects, on both reaction rate and enzyme inactivation; membrane permeability, which was described by the film theory model; and the rejection characteristics of the membrane, which were determined experimentally. However, there were slight losses in enzyme activity during processing.

Other enzymic reactions which have been investigated include the hydrolysis of cellulose, soy protein, fish protein and casein (Cheryan, 1986). A semi-continuous process for lignocellulose hydrolysis has been described by Ishihara *et al.* (1991).

In these long-term continuous processes it is important to minimise enzyme losses and in some processes, to retain, recover or regenerate important enzyme cofactors. Cofactors are required by many enzymes for activity and are classified as prosthetic groups, coenzymes and metal ions. They include compounds such as NAD, FAD, ATP, Co-A. Prosthetic groups are distinguished from coenzymes by their tighter binding to the enzyme, although there is considerable overlap in binding affinities of these two groups (Parkin, 1993).

The cofactors may be immobilised or entrapped with the enzyme or retained within the system by selecting a membrane with a low molecular weight cut-off, which will totally reject them. Another approach is to use negatively charged membranes, which also give rise to higher rejections due to electrostatic interactions. Those which have mainly been investigated are the nicotinamide (NAD and NADP) cofactors.

Another approach is to regenerate cofactors by coupled reactions. Examples investigated include mannitol production from fructose, by mannitol dehydrogenase. Here the NADH produced is regenerated by a coupled reaction involving a glucose dehydrogenase reacting on glucose to produce gluconic acid. The coenzyme turnover number was reported to be 150 000 (Kulbe *et al.*, 1990).

Membrane fermenters

Fermentation processes can also be batch or continuous. Even with continuous processes, the conversion rate may be limited by end-product inhibition and the dilution rate must be less than the specific growth rate of cells, to avoid cell washout. Therefore, another application of membrane reactors is as part of a fermentation vessel, with containment of microorganisms and continuous removal of products. Again the microorganisms can be entrapped or immobilised, typically in the hollow fibre system, or simply allowed to circulate freely with the recycle broth. The freely circulating systems seem to perform more effectively than the immobilised systems. This system operates like a continuous system, with the additional feature that the cells are returned to the fermenter and retained within the system. It is possible to achieve high cell densities and high dilution rates, without the worry of washing out the cells. However, they have not been as thoroughly investigated as enzyme reactors.

One of the most popular applications is the production of alcohol. Cheryan (1986) compares the performance of batch fermentation processes, with continuous culture, immobilised cells and membrane recycle. The membrane recycle gives the biggest productivity in terms of mass of ethanol per unit volume and time. He has also described the use of a membrane reactor to produce alcohol from concentrated whey permeate, using a very high cell density which is retained by the membrane. Such a system has shown a high productivity and has been operated continuously for up to 10 days.

Long-term stability needs to be established. Teixeira *et al.* (1990) used lactose as the carbon source and found that ethanol productivity was 12.5 times higher than continuous fermentation and was directly proportional to the dilution rate. Biomass concentration showed a linear relationship with dilution rate and the largest concentration was eight times that obtained in a conventional continuous fermenter. Endo *et al.* (1990) describe a tubular bioreactor for high-density cultivation of microorganisms, used in the primary part of the circuit. They found improved cell density by over 10 times compared with a conventional fermenter and improved productivity compared to operating at constant dilution rate. Crespo *et al.* (1990) studied the effects of recirculation rate for a propionic acid bacterium fermentation process, linked to a tubular UF module, on permeate flux, fermenter homogeneity, energy consumption, temperature rise and cell damage. High circulation rates improved flux and the degree of homogeneity, but increased cell damage. Energy input was also estimated to determine the degree of cooling required. Propionic bacteria are useful for the production of propionic acid, cyanocobalamin, flavours and dairy starter cultures. Minier *et al.* (1990b) combined the membrane process with distillation, and found that the combination was useful to further concentrate the alcohols and to remove the low molecular weight inhibitors. A mineral ultrafiltration membrane has been used to separate lytic enzymes produced extracellularly during an acetonobutylic fermentation process. The membrane retained the cells but allowed the lytic enzymes to permeate. The permeate was heat treated to inactivate the enzymes, before being returned to the fermenter (Minier *et al.*, 1990a). However, although lytic enzyme activity was decreased by over 6 times, there was no marked increase in the yield of butanol compared to the control.

A novel method of immobilisation involves sandwiching the cells between a reverse osmosis and ultrafiltration membrane. The UF membrane allows free passage of the nutrients to the cells. The RO membrane helps immobilise the cells and permits separation of the alcohol from the sugars and salts. This system has been investigated experimentally and modelled by Jeong *et al.* (1991).

There also exists the possibility of cultivation of animal and plant cells in bioreactors. The performance of a hollow fibre immobilised system has been investigated for the production of antibodies (Piret and Cooney, 1990). Procedures which reduced cell and protein distribution on the shell side of the hollow fibre membrane, led to an improvement in productivity. Ultrafiltration membranes provide a number of advantages, including the retention of growth factors and the selective concentration of high molecular weight protein products.

Recovery of components and downstream processing

Ultrafiltration is now widely used for the recovery of cells, enzymes and other metabolic products. Ultrafiltration and microfiltration can be used for concentrating and harvesting cells but will be in direct competition with continuous centrifugation and to a lesser extent, conventional filtration. The cells may also be shear sensitive, so this factor needs some consideration. Factors such as membrane geometry, pore size, chemical nature of the membrane and ultrastructure should be investigated. Fouling is not necessarily related only to pore size, but to the distribution of pore size, the relationship between pore size and particle size, and whether fouling occurs on the surface or within the pores. Cheryan

(1986) concludes that the overall economics favour membrane processes for small-scale operations, for all particle sizes. However, for larger-scale operations, the economics may favour centrifuges with larger particles, e.g. yeast cells, but the membrane systems for smaller particles.

Ultrafiltration is widely used commercially for concentrating enzyme solutions. There are also many diafiltration operations, for washing out low molecular weight contaminants. For example, Olsen *et al.* (1990) describe the recovery of four enzymes, alkaline phosphatase, hyaluronoglucuronidase, chitinase and beta-N-acetylglucosaminidase, from shrimp waste. Flocculation was induced by ferric chloride at pH 7. The supernatant was then ultrafiltered to recover 99% of the enzyme activity. However, fractionation of the enzymes was not investigated. Pacheco-Oliver *et al.* (1990), used diafiltration to remove coloured impurities from a lipase enzyme, produced by fermentation.

There has been much less success for fractionating enzymes or other components, based solely on size difference, unless there is at least a tenfold difference in their molecular weights. Again the selection of membranes is very important, to minimise excessive enzyme loss in the permeate.

However, there has been some success at partial fractionation and purification, by using compounds which may either bind with one of the fractions and alter its molecular weight and hence its rejection characteristic, or by contacting it with a specific component with which it has a strong affinity. This other component could be introduced in the feed or immobilised within hollow fibre membrane systems. Thus, in conjunction with affinity or ion exchange gels or resins, a specific component in the crude extract can be bound and separated, and subsequently eluted. Examples are given for the purification of horse serum cholinesterase and cattle liver carboxylesterase by these types of method, using an affinity gel and DEAE sephadex, respectively (Molinari *et al.*, 1990). Trypsin has been purified by affinity chromatography and ultrafiltration, using a water-soluble ligand-bound polymer. This was incubated with the crude extract, forming a soluble polymer–trypsin complex. Unbound enzymes were removed by ultrafiltration, whereas the complex was retained. Trypsin was then eluted from the polymer and the eluant was subject to ultrafiltration to further purify the trypsin (US patent, 1990). A French patent (anon) (1991) describes the isolation and recovery of a wide range of enzymes from waste water treatment sludge. Ultrafiltration is used to concentrate the liquid phase, which results from the sludge treated by a number of methods.

Another development is the attachment of bacteria with outer layers rich in glycoproteins to microfiltration membranes. These are then used as a support for protein immobilisation, or as an ultrafiltration membrane (Sara *et al.*, 1990).

It is also possible to separate components such as dissolved organic materials or metal ions by micellar enhanced ultrafiltration (MEUF). The compounds are solubilised within micelles by the addition of surfactants and subsequently removed by ultrafiltration. The processes and factors affecting the flux and rejection of surfactants during ultrafiltration and reverse osmosis are reviewed by Akay and Wakeman (1993).

4.5.5 Medical applications: serum fractionation

One important medical application is for treatment of patients suffering kidney failure, which leads to an accumulation of toxic components, such as urea and creatine within the

blood. There are several treatment choices available: peritoneal dialysis, haemodialysis, haemofiltration and haemodiafiltration (de Burgh, 1992). Peritoneal dialysis makes use of the natural membrane in the peritoneum. A glucose solution is infused into the peritoneal cavity, and solutes and water from the blood diffuse through this membrane into the sugar solution. The sugar solution is infused and removed at hourly intervals. No direct access to the blood supply is required. The other three applications use a manufactured membrane to achieve the removal, with blood being supplied to the membrane. Haemodialysis is usually a short-term, intermittent process, of 2–4 h duration, which requires the availability of dialysis trained nursing staff, whereas haemofiltration and haemodiafiltration can be used continuously for up to several days. Haemodialysis involves the removal of plasma water, containing toxins, through the membrane filter, which is usually either a flat plate or hollow fibre configuration. This filtrate is replaced with a sterile infusion fluid to maintain the patient's fluid status. Where sufficient blood pressure is present, the blood flow can be driven by the patient's own arterial pressure, using either an arterio-venous shunt or an arterial groin line. Where blood pressure is insufficient, a blood pump may be incorporated. Whichever technique is used, the utmost care should be taken to ensure that blood flow is maintained and the filter does not become blocked. Haemodiafiltration uses the same blood circuit, but incorporates a dialysate solution, which is passed through the filter on the permeate side of the membrane, thereby facilitating toxin removal by diffusion. Control of fluid removal may be achieved by adjusting the output of dialysate from the filter in relationship to its input flow. The operating conditions and performance of these two systems are reviewed by Kox and Davies (1992). Further discussion on the use of membranes in artificial organs is given by Leonard (1987).

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Chapter 5

Microfiltration

A. S. GRANDISON, Department of Food Science and Technology, The University of Reading, Reading RG6 6AP and **T. J. A. FINNIGAN**, Marlow Foods, Middlesbrough.

5.1 INTRODUCTION

Microfiltration (MF) is the oldest membrane technology, having been used several decades before the first industrial use of reverse osmosis (Glimenius, 1985). However, subsequent development of the technology has been slow. Until recently microfilters were operated in the dead-end mode and were exclusively of the depth-filter type in which particles become trapped within the filter structure, but recent developments have led to membrane-type microfilters, with a narrow pore size distribution, which can be operated in the cross-flow mode. This has led to an increase in possible applications, including clarification of fluids in the food and beverage industries, recovery of cells and cell debris in the biotechnology industries, and the treatment of wastes.

5.2 THEORY, MATERIALS AND EQUIPMENT

Like ultrafiltration (UF), MF is a pressure-driven process employing pressures considerably lower than reverse osmosis. In fact the distinction between UF and MF is somewhat arbitrary and there is no distinction on purely theoretical grounds. The distinction lies in the size ranges of the materials which are separated. UF is considered to involve the processing of dissolved macromolecules, while MF involves separation of dispersed particles such as colloids, fat globules or cells. MF can be considered to fall between UF and conventional filtration, although there is overlap at both ends of the spectrum. A guide to the pore sizes used for MF could be 0.01–10 μm.

For many years, MF has been applied as a dead-end operation using highly microporous symmetric membranes of the depth-filter type. Such membranes retain particles and consequently result in the build-up of a filter cake. This reduces flow, and when the pressure drop has reached a certain level the membrane must be replaced or removed and regenerated. In addition, the presence of a filter cake radically alters the filtering characteristics, effectively acting as a prefilter which removes particles which

could otherwise pass through the membrane itself. On a large scale, therefore, it is only practicable to use this technique when small amounts of particles are present.

Cross-flow MF (CMF) is a development which combines the cross-flow technique, as applied to UF and reverse osmosis, with MF. CMF can be used to minimise (although it should be emphasised, not completely eradicate) the problems encountered in dead-end MF, and thus permit the processing of fluids containing quite large amounts of suspended solids on a large scale. The advantages result from the fact that the build-up of filter cake is avoided due to the shearing effect of the feed stream flowing parallel to the membrane (Fig. 5.1). CMF plants can be operated in the same batch or continuous modes as described in Chapter 3.

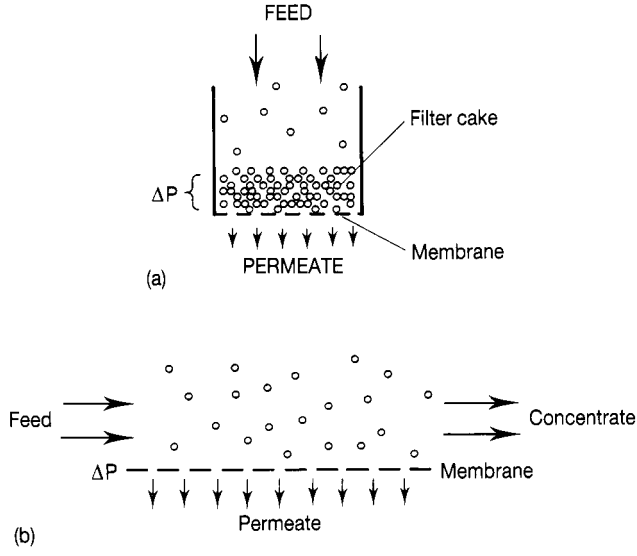


FIG. 5.1. Principles of (a) 'dead-end' and (b) 'cross-flow' filtration.

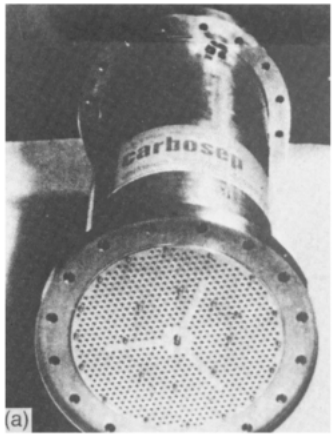
5.2.1 Membrane configurations and characteristics

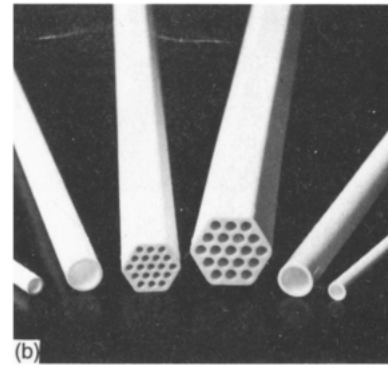
The geometric designs of MF membranes are the same as for UF as described in Chapter 3. Hence the module housings and ancillary equipment are also similar. Also the membrane types are the same as for UF, i.e. cellulose and synthetic polymers (described in Chapter 3) or inorganic. It is notable that the development of inorganic membranes has been towards applications in MF rather than UF and reverse osmosis. In fact some types of inorganic membrane are only available with pore sizes in the MF range.

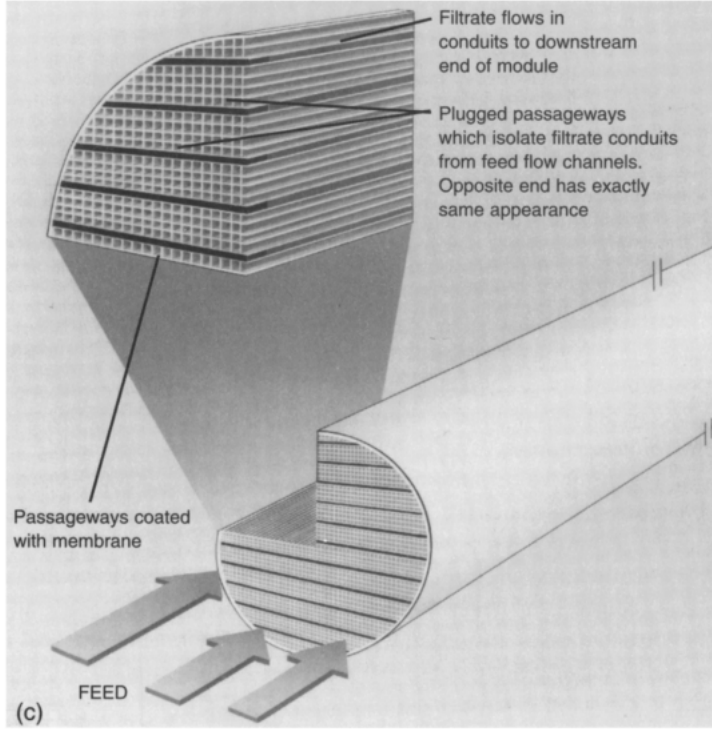
Various inorganic materials have been employed for membrane manufacture including glass, metals and compounds of aluminium, zirconium and titanium, and the geometries can vary radically from conventional membrane design.

The structures and methods of manufacture of inorganic membranes are described in greater detail by Rios *et al.* (1989). Inorganic membranes consist of two parts—a macroporous support and the active membrane coated onto the surface. The supporting

materials must drain away the permeate without any hydrodynamic resistance, and thus have a pore diameter of about 10 μm or more. They are produced from sintered fine powdered materials including alumina, carbon, stainless steel and nickel. The tubular or multichannel geometries of modules (e.g. Fig. 5.2 (a–c)) are produced by extrusion of the






Filtrate flows in conduits to downstream end of module
Plugged passageways which isolate filtrate conduits from feed flow channels. Opposite end has exactly same appearance
Passageways coated with membrane
FEED
(c)

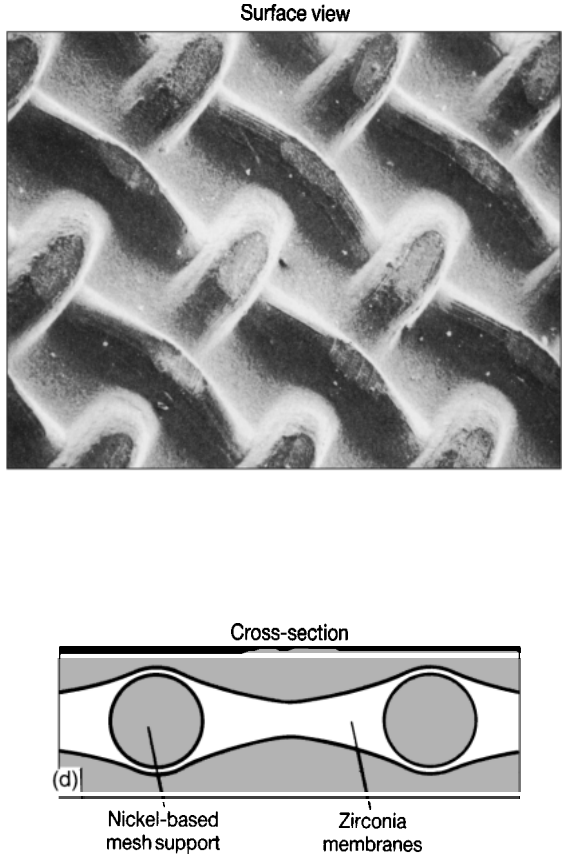


Fig. 5.2. Some designs of inorganic membranes: (a) CARBOSEP membrane composed of zirconia on a carbon support (courtesy of SFEC); (b) different designs of Ceraver alumina membranes (reproduced by permission of Membralox®); (c) alumina membrane module (reproduced by permission of CeraMem); (d) ceramic/metal-mesh composite membrane (reproduced by permission of NWW Acumem Ltd).

powder and binder in aqueous media. The membrane layer may be coated directly onto the macroporous support where pore size is quite large, but for UF and the smaller pore-size MF membranes an intermediate sintered, ceramic layer is necessary due to the surface rugosity of the support. The membrane layer (usually composed of alumina, titania or zirconia) is formed by coating the support with a colloidal suspension and firing at a lower temperature than the firing temperature of the support. To prevent rapid flux decline, the membrane thickness must not exceed a few microns – titanium and zirconium membranes of thickness 3–5 μm have been achieved. Accurate control of the colloidal particle size allows the possibility of producing membranes with an extremely narrow pore size distribution compared to conventional membranes. The final pore size is also related to the sintering temperature. An example of the structure of a sintered ceramic membrane is shown in Fig. 5.3.

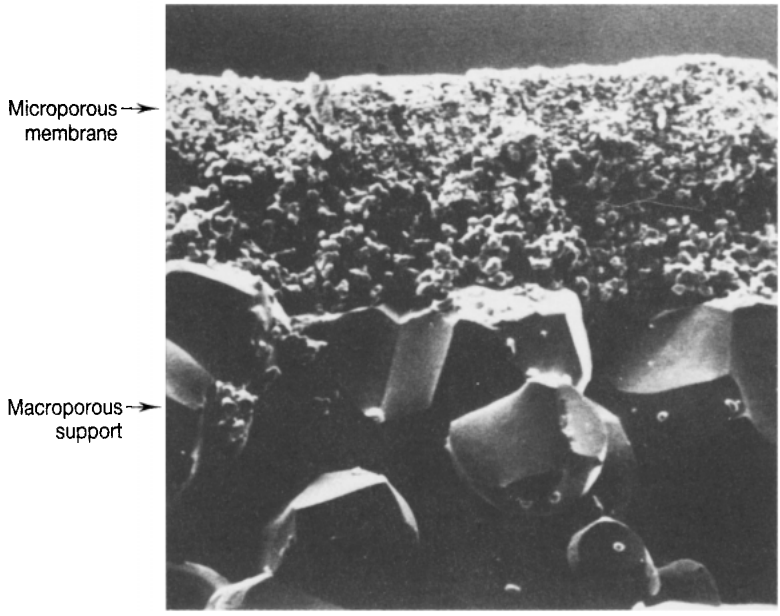


Fig. 5.3. Electron micrograph showing structure of Ceraver sintered alumina membrane with 0.2 μm pore size (reproduced by permission of Membralox®).

Early work on inorganic membranes used glass, but the first reliable cross-flow system was the CARBOSEP membrane made of a microporous layer of zirconia coated onto a macroporous carbon support (Fig. 5.2(a)). New products have subsequently appeared including several designs of alumina membrane (e.g. Ceraver and CeraMem designs – Figs. 5.2(b) and 5.2(c)). A novel design is the composite membrane produced by Acumem composed of a zirconia ceramic membrane with nickel-based superalloy mesh support (Fig. 5.2(d)).

The potential advantages of inorganic membranes result from their greater structural strength and resistance to abrasive degradation, as well as improved chemical and temperature properties. Their rigidity and strength allow the processing of feed materials, such as cheese curd (Mahaut *et al.*, 1982) or particulate materials which would not be possible using conventional designs. Another possibility is that they could be used in conjunction with fluidised turbulence promoters to increase permeate flux, which would be out of the question with the more fragile surfaces of organic membranes. The wide pH ranges of inorganic membranes (e.g. alumina membranes are resistant to pHs ranging from 0.5 to 13.5, although phosphoric and hydrofluoric acids should be avoided) are a great advantage during cleaning and sterilisation. In-place cleaning regimes using high concentrations of caustic soda (3%), nitric acid (2%) and sodium hypochlorite are possible. The modules can withstand temperatures of several hundred °C, which is far beyond the temperatures used for food processing. However, this is an advantage during cleaning cycles and the modules can be sterilised by steam. In practice, operating temperatures are limited by other components such as gaskets, but it could be feasible to

employ high temperatures to defoul individual inorganic membrane modules. The major disadvantage of inorganic membranes is that they are considerably more expensive than polymeric membranes, and while they have some potential advantages, their performance is still limited by concentration polarisation and fouling as with conventional systems.

MF has also been described using membranes produced from woven fabrics or metals.

5.2.2 Performance of microfiltration systems and membrane fouling

The description of flux rate and factors affecting flux rate in UF systems, presented in Chapter 4, applies equally to CMF systems. Engineering aspects and detailed modelling of MF systems are beyond the scope of this chapter, but have been dealt with elsewhere (e.g. Davis and Birdsell, 1987; Ripperger, 1988). In general the larger pore sizes of MF membranes allow very high initial permeate fluxes, although these can decline very rapidly during processing. The flux decline progressively lessens until a near equilibrium filtration rate is observed in most cases (see Fig. 5.4). At this point the shearing action of the cross-flow stream prevents further deposition of material. It is generally assumed that this is actually a state of dynamic equilibrium where particles are leaving and joining the concentration polarisation/fouling layer at the same rate. For any given application it is necessary to carry out experimental trials to establish the most suitable membrane type, and to optimise the pressure and flow characteristics.

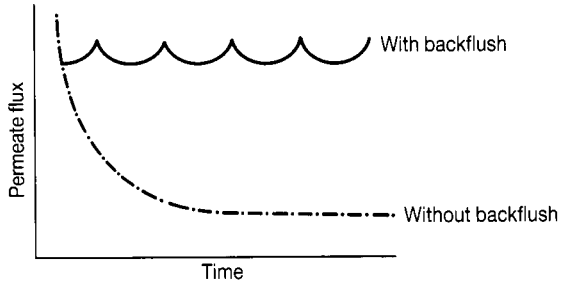


Fig. 5.4. Permeate flux and the effect of backflushing.

As with all membrane processes the permeate flux during MF is limited by concentration polarisation and fouling (see Chapter 4), even with CMF where the formation of filtration cake is prevented or limited. Deep bed clogging of the pores is a particular problem during MF, due to the larger pore sizes. A paradoxical situation can occur in CMF whereby flux may be maintained at a higher level when a tighter membrane (i.e. with lower pore size) is used. This arises when the pores are smaller than the suspended solids in the feed, so that the particles cannot penetrate the pores. An example of this was described by Gatenholm *et al.* (1988), who compared MF with UF membranes for harvesting *Escherichia coli* cells from fermentation broths. The MF membranes showed such a high initial flux decline that the steady state flux was actually higher for the UF than MF. Also Le and Atkinson (1985) noted that permeate flux during CMF of cell suspensions using membranes with a range of pore sizes followed the order 0.45 μm > 0.6 μm > 0.2 μm with respect to pore size. Clearly there is a need to choose

membranes of the appropriate pore size in relation to the characteristics of the particles suspended in the feed. Unfortunately it is very difficult to predict the optimal pore size from first principles, and experimentation is required. However, a recent study of Tarleton and Wakeman (1993) has attempted to understand the relationship between pore size and particle size in flux decline. Clearly the particle size distribution is an important factor, especially when even a small proportion of fines are present in the feed. Many anomalies remain, and further systematic studies are required.

A large amount of effort has been put into devising means of avoiding or counteracting the effects of fouling in MF, and several promising techniques have evolved. The first requirement is to maintain high shear in the retentate and thus reduce the thickness of the boundary layer at the membrane surface and remove deposits from the membrane. It is desirable that turbulent flow be maintained on the retentate side of the membrane. Murkes (1989) has described a new high shear cross-flow method for MF, and claimed an order of magnitude increase in flux rates over conventional CMF.

Forcing a quantity of permeate back through the membrane (i.e. backflushing) can drive deposited particles away from the membrane surface, as well as breaking up concentration polarisation and/or gel layers in the bulk flow. This can lead to regeneration of permeate flux, but it is necessary to ensure that a higher volume of permeate is not used for the backflushing than is gained from the resulting increased permeate flux (Milisic and Bersillon, 1986). However, assuming the correct sequencing and volume of pulsations are used, the technique can be most beneficial (Fig. 5.4). An alternative method of backwashing is to use cleaning sequences consisting of blowing air through the membranes – i.e. gas backflushing (Dietrich *et al.*, 1988; Peters, 1989). This may prove beneficial with problem feed streams. Another approach to maintaining flux is to use the 'uniform transmembrane pressure' mode of operation. This requires simultaneous operation of a retentate pumping loop and a permeate pumping loop, adjusted so that the pressure *drop* across the membrane is small, and is uniform along the length of the membrane. This system has allowed very high fluxes (500–700 $l\ m^{-2}\ h^{-1}$) to be maintained during processing of skimmed milk using ceramic membranes in the 'Bactocatch' system (see Section 5.3.1).

The application of an electric field has been shown to reduce fouling due to colloids and particles during CMF (Visvanathan and Ben Aim, 1989) and thus prevent flux decline (Tarleton and Wakeman, 1988), although the mechanism of action is not fully understood. Bowen *et al.* (1989) reported that electrical pulses were effective in both improving flux rates and restoring flux rates in fouled systems, and could be used as an alternative to backwashing and conventional cleaning techniques.

Other approaches to reducing fouling during MF have included the use of abrasives to break down the fouling layers, and the application of pulsations to the feed stream (Milisic and Bersillon, 1986). Chemical pretreatments of the feed streams can also be used to reduce the problems of fouling (Bedwell *et al.*, 1988), although this is frequently not a suitable option. Pretreatment of the membrane may reduce the flux decline during fouling. Taddei and Howell (1989) reported a 70% improvement of flux during harvesting of yeast cells by conditioning the MF membranes with respect to pH.

The initial challenge to the membrane has been found to affect the subsequent flux during MF. Care must be taken during start-up procedures to prevent too-rapid flux

decline. It is frequently an advantage to start the run in the dynamic mode (i.e. with water circulating prior to introduction of the feed), or with the permeate side filled with solvent.

While the above methods of combating fouling can be very effective, it is essential to combine them with efficient chemical cleaning procedures.

A characteristic of MF is that the separation may alter during processing due to fouling, such that the MF operation, in reality, becomes UF through a 'secondary membrane' formed at the surface of the MF membrane, consisting of macromolecules or colloids. This generally constitutes a problem. However, this property has been used to advantage in the French dairy industry for the concentration of milk. Bennassar *et al.* (1982) described the use of the selective UF properties of the secondary membrane formed while using 0.2 μm membranes. Almost total retention of milk protein was possible with this membrane.

5.3 APPLICATIONS IN THE FOOD AND BIOTECHNOLOGY INDUSTRIES

MF is generally used to separate particles suspended in liquid media, and may frequently be considered as an alternative to conventional filtration or centrifugation. For industrial use the aim is usually to obtain either a clear permeate or the concentrate. Therefore most applications are either clarification, or the recovery of suspended particles such as cells or colloids, or the concentration of slurries. MF is a useful process for the treatment of fermentation broths, frequently as an alternative to centrifugation. During recovery of intracellular components, the cells can be enriched and washed by diafiltration prior to disintegration of the cells, and cell debris can be removed from the products of lysis.

MF membranes can be incorporated in water polishing systems as they are effective in removing suspended solids and bacteria. They may also be applied to a variety of liquid food streams, and may give the accompanying benefit of chemical and/or microbiological stabilisation. Clarification of biochemical or microbiological reaction products or effluents is also possible. MF is a well-established laboratory technique for the production of sterile fluids without the application of heat.

5.3.1 Food industry

Perhaps the majority of applications in the food industry have been in the treatment of juices and beverages. As MF is a purely physical process, it can have advantages over traditional methods involving chemical additives, in terms of the quality of the product as well as the costs of processing.

Finnigan and Skudder (1989) discussed the application of ceramic microfilters to the processing of beer and cider. Very good quality clear permeate was found for both products with high flux rates. Fluxes of 200–400 l m^{-2} h^{-1} were obtained with beer at low temperatures with no rejection of essential components. Recovery of beer from tank bottoms is also possible using ceramic MF, but this is less important in the UK now that duty is not paid at the wort stage of production.

Clarification and biological stabilisation of wine musts and unprocessed wine have been described. This avoids the requirement for fining and, possibly, pasteurisation. Dau *et al.* (1988) used membrane filters with pore size 0.1–5 μm to clarify wine preheated to

30–40°C to increase permeate flux. However, Rios *et al.* (1989) selected ceramic membranes with pore diameter 0.2 μm at temperatures <20°C to avoid sensory changes.

Conventional methods of clarification of fruit juices incorporate the use of diatomaceous earth and enzymes to remove a variety of materials including suspended solids, colloidal particles, proteins and condensed polyphenols. Also they are quite time consuming in that the juice must be stored in settling tanks. Juice clarification using MF is becoming an established commercial practice as it is a continuous process which can result in significant savings in time, materials and labour as well as improved yields. (*Note*: The use of diatomaceous earth results in loss of yield.) Short (1988) described the commercial application of CMF to the processing of a range of fruit juices. A plant producing more than 5×10^5 litres per day of apple juice exists in the USA. This has the major benefit of increasing juice yield from 95 to 97% as well as eliminating the requirement for diatomaceous earth as a pretreatment, and the use of enzymes and fining agents. A saving of $350 000 per annum was estimated. With highly coloured juices, a further benefit of MF is that the colour is retained very well in the permeate. Commercial systems are also known to exist for grape, pear, kiwifruit, pineapple, cranberry and citrus juices. Clarifications of vinegar, vodka, pickling brines and liqueurs by MF have also been described (Le and Billigheimer, 1985; Short, 1988).

It seems likely that CMF could replace carbonatation in the treatment of raw sugar beet juice, although this is not carried out commercially to the author's knowledge. Another section of the industry with several applications is dairy processing. Piot *et al.* (1984) and Merin (1986) have clarified sweet cheese whey using CMF. This has the dual benefit of removing fat and reducing the bacterial population and could eliminate the need for fat separation and heat treatment in the production of whey protein powders prior to UF. The former authors reported that a decimal reduction of microorganisms of 5 could be obtained in the microfiltrate compared to the whey, although some loss of whey protein was observed. Hanemaaijer (1985) described a scheme for whey treatment incorporating MF and UF to produce 'tailor-made' whey products with specific properties for specific applications. The products include whey protein concentrates which are rich in whey lipids, as well as highly purified protein.

Bacterial removal from whole milk by MF is a problem because the size range of the bacteria overlaps with the fat globules, and to a lesser extent with the casein micelles. However, some success has been achieved with skim milk. The 'Bactocatch' system can remove 99.6% of the bacteria from skim milk using ceramic membranes on commercial scale (Malmbert and Holm, 1988). The retentate (approximately 10% of the feed) can then be sterilised by a UHT process, mixed with the permeate and the mixture pasteurised, to give a product with 50% longer shelf-life but no deterioration in organoleptic properties compared to milk that has only been pasteurised. The combination of MF and heat reduces the bacterial numbers by 99.99% (4D). Alternatively the permeate could be used for cheesemaking, or the production of low-heat milk powder (Hansen, 1988). Piot *et al.* (1987) described the use of membranes of pore diameter 1.8 μm to produce skim milk of low bacterial content. Recovery of fat from buttermilk has also been described (Rios *et al.*, 1989).

Membranes have been used to concentrate milk prior to the manufacture of many cheese types. This results in improved yields and other associated benefits such as

reduced requirement for rennet and starter, and the ability to produce much more cheese per vat (Grandison and Glover, 1994). In most cases UF membranes have been used as it is necessary to retain all the protein in the concentrate. An alternative approach is to concentrate the curd after coagulation of the milk in which case a solution of lactose and minerals is removed from the semi-solid protein gel. This can be done using centrifugation in the manufacture of some soft cheese types.

However, the use of MF is an attractive alternative. Rios *et al.* (1989) have carried out extensive trials on this application and concluded that the use of 0.2 μm pore diameter membranes gave a product with better texture and yield than with centrifugation. The choice of ceramic membranes allowed the curd to be contacted directly with the membrane.

Other food applications have been reported with meat and vegetable products including the following.

Devereux and Hoare (1986) described the use of MF to recover precipitated soya protein. This could have advantages over recovery of the dissolved protein using UF.

Gelatin is a proteinaceous material derived by hydrolysis of collagen. This is purified by filtration incorporating diatomaceous earth. The latter process can be replaced by CMF which effectively removes dirt, coagulated proteins, fats and other particulate materials from the feed. Again the CMF method gives higher yields of high quality product on a continuous basis. Short (1988) calculated that incorporating CMF plants for gelatin would have a payback time of 3 years for a capacity of 30 tonnes/h.

5.3.2 Applications for biotechnology

Applications of MF in the biotechnology industry are very promising although the process has not yet made the breakthroughs that may have been expected. The major problem with MF of suspensions of cells or cell debris is the exponential flux decay resulting from adhesion of cells or cell fragments to the membrane – i.e. biofouling. This has limited the application of CMF into biotechnological downstream processing. Solutions to this problem, other than general methods of avoiding fouling, are discussed by Defrise and Gekas (1988) and include choice of biocompatible membrane materials and the use of surfactants and polyelectrolytes. Most biotechnological applications of MF are as a competitor to centrifugation, and MF is becoming recognised as a viable alternative in many cases. CMF may have advantages over centrifugation when containment is required (e.g. when handling pathogenic organisms or in recombinant DNA technology) as aerosols are not produced during the operation (Kroner *et al.*, 1984). The capital and maintenance costs of MF are lower than for centrifugation, although membrane replacement is expensive. Tutunjian (1985) compared the costs of process-scale harvesting and washing of *Escherichia coli* cells using MF with a hollow fibre system to centrifugation. The MF system had about 70% lower capital, and 25% lower running costs than centrifugation. Also virtually 100% recovery of solids is possible with MF, whereas it is often less than 90% with centrifugation.

CMF is a versatile technique for cell harvesting and cell debris removal during recovery of intracellular products. The viability and enzyme content of the cells is unaffected by MF, and it is possible to concentrate cell suspensions to 20–25% dry weight, limited by pumping considerations (Le and Atkinson, 1985). A 0.1 μm

microporous hollow fibre membrane has been used for the purification of bovine growth hormone from *Escherichia coli* lysate (Tutunjian, 1985). Although the growth hormone was partially rejected from the membrane, this could be recovered using diafiltration such that over 99% recovery from the original lysate was possible. Kroner *et al.* (1984) used MF to recover a range of soluble enzymes from cell debris, although they concluded that the separation was not satisfactory due to high retention of the enzymes in the feed. Transmission of enzymes through microporous membranes is rather anomalous. Le and Billigheimer (1985) found that transmission of arylamidase increased rapidly over the first 5–10 minutes of processing and then declined steadily. This was ascribed to adsorption of protein to the membrane surface during the initial phase, to form a monolayer lining. Transmission of the enzyme occurred when the surface was saturated, and subsequently declined due to the formation of a secondary membrane of cell debris and proteinaceous matter. In general, careful manipulation of pressures and flow rates can be used to optimise protein transmission through MF membranes.

Alternatively, valuable extracellular biotechnological products can be separated from cell suspensions by MF. Raehse *et al.* (1986) employed polysulphone tubular membranes with a pore size of 0.3–0.5 μm to separate alkaline protease from a fermentation broth. The ratio of the mean pore diameter of the membrane to the size of the microorganisms was between 0.15 and 0.85, which gave a rapid separation suitable for large-scale applications.

A combination of CMF with affinity chromatography provides an innovative development for purifying biochemicals from fermentation broths or other aqueous media, such as trypsin from pancreatic extract. The principle is to bind the biochemical to a macroligand and remove contaminants by CMF. Following dissociation, the purified material can then be recovered by CMF, and the macroligand recycled (Luong *et al.*, 1987).

It is possible to incorporate MF membranes into membrane reactors. In this way a continuous process can be developed in which the membranes are retentive to the cells (or other biocatalyst), but permeable to the reaction products. Most reported developments in this field have been on laboratory scale, and use UF rather than MF membranes. One promising report with MF is the incorporation of zirconia membranes into a continuous fermentation system with cell recycle for production of alcohol which permitted very high yeast concentrations to be used (Lafforgue *et al.*, 1987). This has led to the possibility of extremely high alcohol production capacity (possibly 150 kg m^{-2} h^{-1}).

5.4 CONCLUSIONS

MF has made significant advances in new applications in the food and biotechnology industries. However, the technique has not yet realised its full potential, largely due to the severe problems of flux decline due to fouling. It is believed that further developments in membrane design and a greater knowledge of fouling mechanisms will result in greater application in the future, especially in the field of downstream processing.

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Chapter 6

Ion-exchange and electrodialysis

ALISTAIR S. GRANDISON, Department of Food Science and Technology, The University of Reading, Reading RG6 6AP, UK

Ion-exchange and electrodialysis are distinct methods of separation, but can conveniently be treated together, as the basic criterion for separation in both cases is the molecular electrostatic charge. While ion-exchange involves retention of ionised solutes on a solid support material, electrodialysis permits the separation of ions using selective ion-exchange membranes.

6.1 ION-EXCHANGE

Ion-exchange methods can potentially be used for separations of many types of molecules such as metal ions, proteins, amino acids or sugars. The technology is utilised in many sensitive analytical chromatography procedures, frequently on a very small scale. On the other hand industrial-scale production operations, such as demineralisation or protein recovery, are possible. This chapter will consider only the larger-scale applications which have current or potential use for production in the food and biotechnology industries.

6.1.1 Theory, materials and equipment

A brief summary of the theory of ion-exchange will be given here. More detailed accounts can be found elsewhere (e.g. Vermeulen *et al.*, 1984; Walton, 1983; Helfferich, 1962).

Solute/ion-exchanger interactions

Ion-exchange could be defined as the selective removal of a single, or group of, charged species from one liquid phase followed by transfer to a second liquid phase by means of a solid ion-exchange material. In practice this involves the process of adsorption – the transfer of specific solute(s) from a heterogeneous feed solution on to the solid ion-exchanger. The mechanism of adsorption is electrostatic, involving opposite charges on the solute(s) and the ion-exchanger. The feed solution is washed off, and this is followed

by desorption, in which the separated species are recovered back into solution in a much purified form.

The ion-exchange solids bear fixed ions which are covalently attached to a solid matrix. There are two basic types of ion-exchanger:

(1) Cation exchangers (sometimes called 'anionic exchangers') which bear fixed negative charges and are therefore able to retain cations, and
(2) Anion exchangers (sometimes called 'cationic exchangers') which bear fixed positive charges.

Ion-exchangers can be used to retain simple ionised species, but may also be used in the separation of polyelectrolytes which possess both positive and negative charges (i.e. amphoteric molecules such as proteins) as long as the overall charge on the polyelectrolyte is opposite to the fixed charges on the ion-exchanger. This overall charge depends on the isoelectric point of the polyelectrolyte and the pH of the solution. At pH values lower than the isoelectric point the net overall charge will be positive and *vice versa*. In some circumstances it is even possible for ion-exchangers to retain macromolecules of like charge, presumably if a portion of the molecule carries a sufficient opposite charge (Peterson, 1970). The main interaction is via electrostatic forces, and in the case of polyelectrolytes the affinity is governed by the number of electrostatic bonds between the solute molecule and the ion-exchanger. However, particularly with large molecules such as proteins, multiple interactions may occur involving steric effects. Size and geometric properties, and the degree of hydration of the ions may affect these interactions, and hence the selectivity of the ion-exchanger for different ions. Charge density may be more important than overall charge in determining the relative selectivity.

Figure 6.1 is a schematic diagram showing a generalised anion exchanger – i.e. bearing fixed positive charges. To maintain electrical neutrality these fixed ions must be balanced by an equal number of mobile ions of the opposite charge (i.e. anions) which are held by electrostatic forces. These mobile ions can move in and out of the porous molecular framework of the solid matrix and may be exchanged stoichiometrically with other dissolved ions of the same charge, and are termed counterions. Ion-exchange systems can be considered to consist of two aqueous liquid phases – one confined within

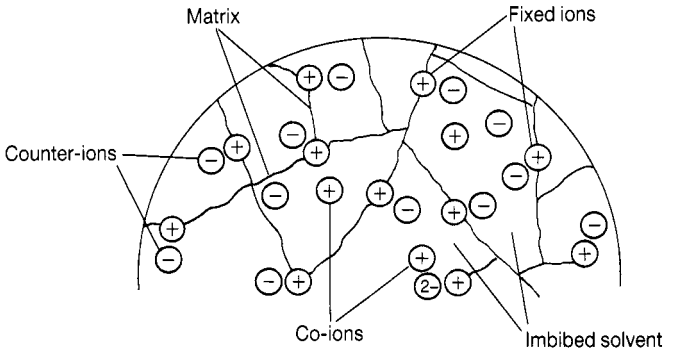

Fig. 6.1. Schematic diagram of a generalised anion exchanger.

the structure of the solid matrix in equilibrium with an outside phase. The interface between the two phases acts as a semipermeable membrane which allows the passage of any mobile ionic species depending on the Donnan equilibrium. This states that the chemical potential of a salt must be the same inside and outside the ion-exchanger – e.g. in the simplest case where the only mobile ions present are Na^+ and Cl^-, then at equilibrium,

$$[Na^+][Cl^-]_{\text{Inside phase}} = [Na^+][Cl^-]_{\text{Outside phase}}$$

Thus a certain proportion of co-ions (mobile ions having the same sign – Na^+ in this example – as the fixed ions) will be present even in the internal phase. Therefore, if an anion exchanger (as in Fig. 6.1) is in equilibrium with a solution of NaCl, the internal phase contains some Na^+ ions, although the concentration is less than in the external phase because the internal concentration of Cl^- ions is much larger.

When an ion-exchanger is contacted with an ionised solution, equilibration between the two phases rapidly occurs. Water moves into or out of the internal phase so that osmotic balance is achieved. Counterions also move in and out between the phases on an equivalent basis. If two or more species of counterion are present in the solution, they will be distributed between the phases according to the proportions of the different ions present and the relative selectivity of the ion-exchanger for the different ions. It is this differential distribution of different counterions which forms the basis of separation by ion-exchange. The relative selectivity for different ionised species results from a range of factors. The overall charge on the ion and the molecular or ionic mass are the primary determining factors, but selectivity is also related to degree of hydration, steric effects and environmental factors such as pH or salt content.

In the adsorption stage, a negatively charged solute molecule (e.g. a protein P^-) is attracted to a charged site on the ion-exchanger (R^+) displacing a counterion (X^-):

$$R^+X^- + P^- \rightarrow R^+P^- + X^-$$

In the desorption stage, the anion is displaced from the ion-exchanger by a competing salt ion (S^-), and hence is eluted:

$$R^+P^- + S^- \rightarrow R^+S^- + P^-$$

Ion-exchangers may be further classified in terms of how their charges vary, with changes in pH, into weak and strong exchangers. The terms strong or weak do not refer to the strength of binding of the ions to the exchanger, or the mechanical strength of the matrix, but to the pH range over which the materials are effective. Strong ion-exchangers are ionised over a wide range, and have a constant capacity within the range, whereas weak exchangers are only ionised over a limited pH range (e.g. weak cation exchangers may lose their charge below pH 6 and weak anion exchangers above pH 9). Thus weak exchangers may be preferable to strong ones in some situations, for example where desorption may be achieved by a relatively small change in pH of the buffer in the region of the pK_a of the exchange group. Regeneration of weak ion-exchange groups is easier than with strong groups, and therefore has a lower requirement of costly chemicals.

Ion-exchange groups

Some common examples of cation exchangers are

$-SO_3^-H^+$ (strong – pK_a 1–2)

$-PO_3^{2-}(H^+)_2$ (medium – pK_a 2–3)

$-COOH$ (weak – pK_a 3.5–8)

Base function is almost invariably present as amines or imines. These are introduced into the matries by chloromethylation, followed by reaction with the appropriate amine to produce weakly to strongly basic ion-exchangers. Some common examples are

$-O-CH_2-CH_2-NH^+-(CH_2-CH_3)_2$ (diethylaminoethyl – DEAE)

$-O-CH_2-CH_2-NH_3^+$ (amino ethyl – AE)

$-O-CH_2-CH_2-N^+(C_2H_5)_2-CH_2-CH(OH)-CH_3$ (quaternary amino ethyl – QAE)

$-CH_2-N^+(CH_3)_3$ (quaternary amine – Q)

Q and QAE are strong anion exchangers while DEAE and AE are weak.

Ion-exchange materials

All ion-exchangers basically consist of a solid insoluble matrix to which are attached the active, charged groups on which ion-exchange occurs. Various terms are used to describe this material including resin, adsorbent, medium, or just ion-exchanger. There is no general agreement on which is correct, and the usage is sometimes confusing – e.g. the term 'resin' is sometimes used as a general term for ion-exchangers, or sometimes specifically for synthetic organic materials, while a resin is strictly a naturally occurring organic compound (Kanekanian and Lewis, 1986).

The solid support must have an open molecular framework which allows the mobile ions to move freely in and out, and must be completely insoluble throughout the process. Most commercial ion-exchangers are based on an organic polymer network, although inorganic materials may be used. The support material does not directly determine the ionic distribution between the two phases, but it is a major factor in determining the physical and chemical stability of the ion-exchanger. Hence this will determine factors such as the capacity, the flow rate through a column, the diffusion rate of counterions into and out of the matrix, the degree of swelling and the durability of the material. The materials tend to be of two main types – xerogels or aerogels. Xerogels are insoluble synthetic polymers containing a cross-linking agent. Their structure and porosity depends on the solvent and degree of solvation and they are compressible to some degree. Xerogels make up the majority of commercially available ion-exchangers including polyacrylamides, polystyrene and dextrans. The pore size of these materials can be controlled by the manufacturing conditions, especially the degree of cross-linking. Aerogels have a much more fixed rigid structure (e.g. porous silica) and are therefore incompressible, which has obvious advantages for production scale.

As the adsorption is a surface effect, the available surface area is a key parameter. For industrial processing the maximum surface area to volume should be used to minimise plant size and product dilution. It is possible for a 1 ml bed of ion-exchanger to have a total surface area >100 m^2. The ion-exchange material is normally deployed in packed beds, and involves a compromise between large particles (to minimise pressure drop) and small particles to maximise mass transfer rates. Porous particles are employed to increase surface area/volume. However, the surface must also be accessible to the solute molecules, and hence materials with an enormous surface area due to the presence of minute pores may be of very limited use, because much of this surface is inaccessible even to small solute molecules. Manufacturers of ion-exchange materials generally quote the exclusion limit of products with respect to molecular size. Particularly in the case of biopolymers, the shape of the pores and the three-dimensional structure of the solute may be a further consideration.

Capacity

The capacity of an ion-exchanger is defined as the number of equivalents of exchangeable ions per kilogram of exchanger but is frequently expressed in meq/g (usually in the dry form), and can be determined by titration of the charged groups with strong acid or base. This property depends on the nature of the fixed ions as well as the available surface area. Most commercially available materials have capacities in the range 1–10 equivalents/kg of dry material.

Blinding and fouling

The operational life of an ion-exchanger, or at least the time between major clean-up campaigns, is limited by blinding or fouling. This is non-specific adsorption onto the matrix surface, or within the pores, which effectively reduces the capacity, and certainly affects the choice of ion-exchanger for a particular separation. The susceptibility of an ion-exchanger to blinding or fouling with a particular feedstock may exclude its use for that function despite having otherwise excellent binding capacity and specificity for the molecules in question. For example, the presence of significant lipid levels in a feedstock may exclude the use of some exchangers for protein separations.

Elution

The choice of method of elution depends on the specific separation required. In some cases the process is used to remove impurities from a feedstock, while the required compound(s) remains unadsorbed. No specific elution method is required in such cases, although it is necessary to regenerate the ion-exchanger with strong acid or alkali. In other cases the material of interest is adsorbed by the ion-exchanger while impurities are washed out of the bed. This is followed by elution and recovery of the desired solute(s). In the latter case the method of elution is much more critical – for example, care must be taken to avoid denaturation of adsorbed proteins.

Elution of the adsorbed solute is effected by changing the pH or the ionic strength of the buffer, followed by washing away the desorbed solute with a flow of buffer.

Increasing the ionic strength of the buffer increases the competition for the charged sites on the ion-exchanger. Small buffer ions with a high charge density will displace

polyelectrolytes which can subsequently be eluted. Altering the buffer pH so that the charge on an adsorbed polyelectrolyte is neutralised or made the same as the charges on the ion-exchanger will result in desorption.

Ion-exchange columns
Fixed bed operations consisting of one, or two columns connected in series (depending on the type of ions which are to be adsorbed), are used in most ion-exchange separations. Liquids should penetrate the bed in plug flow, in either downward or upward direction. The major problems with columns arise from clogging of flow and the formation of channels within the bed. Problems may also arise from swelling of organic matrices when the pH changes.

Mixed bed systems
These may be used to avoid prolonged exposure of the solutions to both high and low pH environments, as is frequently encountered when using anion and cation exchange columns in series (e.g. during demineralisation of sugar cane juice to prevent hydrolysis of sucrose as described below). Cation and anion exchangers are intimately mixed during the adsorption phase so that the feed solution remains at high or low pH only for the time required to pass from one particle to the next. Regeneration is possible on the basis that the two exchange materials have different specific gravities, and thus separate into two layers on backwashing. By the use of a regenerant distributor, strong acids and alkalis may be used to regenerate the resins independently. After rinsing, the ion-exchangers are remixed using compressed air.

Stirred tanks
The flow and swelling problems encountered with fixed beds are obviated by the use of stirred tanks; however, these systems are less efficient and expose the ion-exchangers to mechanical damage as there is a need for mechanical agitation. The system involves mixing the feed solution with the ion-exchanger and stirring until equilibration has been achieved (typically 30–90 min in the case of proteins – Kanekanian and Lewis, 1986). After draining and washing the ion-exchanger, the eluant solution is then contacted with the bed for a similar equilibration time before draining and further processing.

6.1.2 Applications of ion-exchange in the food and biotechnology industries

One method of classifying the applications of ion-exchange could be by industries or commodities. The main areas of the food industry where the process is currently used or is being developed are sugar, dairy and water purification, although sufficient applications occur outside these to render this classification unsatisfactory. Ion-exchange is widely employed in the recovery, separation and purification of biochemicals, monoclonal antibodies and enzymes.

Another way of categorising the applications is by the type of separations attained, for example:

(1) removal of minor components, e.g. deashing or decolorising;
(2) enrichment of fractions, e.g. recovery of proteins from whey or blood;
(3) isolating valuable compounds, e.g. production of purified enzymes.

Alternatively the chemical nature of the adsorbed ions could be used as a basis for classification. Any ionisable component of a foodstuff can potentially be adsorbed on to an ion-exchanger and thus separated.

The following is an attempt to classify applications in food and biotechnology on the basis of the function of the process.

Softening

Softening of water and other liquids involves the exchange of calcium and magnesium ions for sodium ions attached to a cation exchange resin, e.g.

$$R—(Na^+)_2 + Ca(HCO_3)_2 \rightarrow R—Ca^{2+} + 2NaHCO_3$$

The sodium form of the cation exchanger is produced by regenerating with NaCl solution. Apart from the production of softened water for boiler feeds and cleaning of food and processing equipment, softening may be employed to remove calcium from sucrose solutions prior to evaporation (which reduces scaling of heat exchanger surfaces in sugar manufacture), and from wine (which improves stability) (Cristal, 1983).

Demineralisation

Demineralisation using ion exchange is an established process for water treatment, but over the last 20 years it has been applied to other food streams. Typically the process employs a strong acid cation exchanger followed by a weak or strong base anion exchanger. The cations are exchanged with H^+ ions, e.g.

$$2R^-H^+ + CaSO_4 \rightarrow (R^-)_2Ca^{2+} + H_2SO_4$$

$$R^-H^+ + Na^+Cl^- \rightarrow R^-Na^+ + H^+Cl^-$$

and the acids thus produced are fixed with an anion exchanger, e.g.

$$R^+OH^- + H^+Cl^- \rightarrow R^+Cl^- + H_2O$$

Demineralised cheese whey is desirable for use mainly in infant formulations, but also in many other products such as ice cream, bakery products, confectionery, animal feeds etc. The major ions removed from whey are Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HPO_4^-, citrate and lactate. Ion-exchange demineralisation of cheese whey generally employs a strong cation exchanger followed by a weak anion exchanger (Houldsworth, 1976). This can produce more than 90% reduction in salt content, which is necessary for infant formulae. Lower levels of demineralisation, obtained using a by-pass system, may be adequate for other applications. Due to the high salt content of whey, the system must be regenerated after the treatment of 10–15 bed volumes of whey. This is achieved, following rinsing, by the treatment of cation and anion exchangers separately with strong acids and alkalis respectively. Typically a cycle is about 6 h, of which 4 h are required for regeneration, therefore two or three parallel systems may be necessary. The use of countercurrent regeneration reduces the consumption of regeneration chemicals.

Jonsson (1984) described the SMR (Swedish Dairies Association) process for whey demineralisation, in which the whey first enters a weak anion column in which the whey anions are exchanged for HCO_3^- ions. Following this a weak cation column exchanges the

whey cations for NH_4^+. The whey salts are thus exchanged for ammonium bicarbonate which decomposes to NH_3, CO_2 and water during subsequent evaporation, the NH_3 and CO_2 being recovered. Jonsson and Arph (1987) compared conventional ion-exchange demineralisation of cheese whey to the SMR process and concluded that the requirement for regeneration chemicals and production of waste chemicals are much reduced in the SMR process.

Demineralisation by ion-exchange resins is used at various stages during the manufacture of sugar from either beet or cane, as well as for sugar solutions produced by hydrolysis of starch. In the production of sugar from beet, the beet juice is purified by liming and carbonatation and then may be demineralised by ion-exchange (McGinnis, 1971). The carbonated juice is then evaporated to a thick juice prior to sugar crystallisation. Demineralisation may, alternatively, be carried out on the thick juice which has the advantage that the quantities handled are much smaller, but is limited by the fact that diffusion rates are low at high sugar concentrations. To produce high-quality sugar the juice should have a purity of about 95%. Rousseau (1984) described the 'new demineralisation/demi' process which utilises a mixed bed of weak cationic and weak anionic resins in a batchwise process to treat the thick juice (dry matter 70%). This gives rise to a very pure thick juice with minimum dilution, with the bonus of a decolorisation at no extra cost. A further application in beet sugar production is the Quentin process by which the sugar level of molasses can be decreased. This is achieved by exchanging potassium and sodium ions of the juice prior to the final crystallisation, for magnesium using a strongly acidic cation exchanger. Magnesium is less molassigenic than alkaline ions.

Ash removal or complete demineralisation of cane sugar liquors has been described by Chen (1985). The process is carried out on liquors that have already been clarified and decolorised, so the ash load is at a minimum. The use of a mixed bed of weak cation and strong anion exchangers in the hydrogen and hydroxide forms, respectively, reduces the prolonged exposure of the sugar to strongly acid or alkali conditions which would be necessary if two separate columns were used. Destruction of sucrose is thus minimised.

The cation and anion resins are sometimes used in their own right for dealkalisation or deacidification, respectively. Weak cation exchangers may be used to reduce the alkalinity of water used in the manufacture of soft drinks (Carney, 1988) and beer (Cristal, 1983), while anion exchangers can be used for deacidification of fruit and vegetable juices (Lue and Chiang, 1989; Dechow *et al.*, 1985). In addition to deacidification, anion exchangers may also be used to remove bitter flavour compounds (such as naringin or limonin) from citrus juices (Johnson and Chandler, 1985). Anion or cation exchange resins are used in some countries to control the pH or titratable acidity of wine (Rankine, 1986; Bonorden *et al.*, 1986) although this process is not permitted by other traditional wine producing countries. Acidification of milk to pH 2.2, using ion-exchange during casein manufacture by the Bridel process, has also been described (Pierre and Douin, 1984).

Ion-exchange processes can be used to remove specific metals or anions from drinking water and food fluids, which has potential application for detoxification or radioactive decontamination. For example, procedures have been described for the removal of lead (Brajter and Slonawska, 1986), barium and radium (Snoeyink *et al.*, 1987), aluminium

(Pesavento *et al.*, 1989), uranium (Sorg, 1988) and nitrates (Lauch and Guter, 1986) from drinking water. Removal of a variety of radionuclides from milk has been demonstrated. Radiostrontium and radiocaesium can be removed using a strongly acidic cation exchanger (Tait *et al.*, 1989; Koga *et al.*, 1968), while I^{131} can be adsorbed on to a variety of anion exchangers (Barth *et al.*, 1970). The production of low sodium milk, with potential dietetic application, has been demonstrated (Nakazawa and Hosono, 1989).

Decolorisation

Demineralisation processes may have the added benefit of colour removal. There are, however, other cases where colour removal is required without demineralisation.

Sugar liquors from either cane or beet contain colourants such as caramels, melanoidins, melanins or polyphenols combined with iron. Many of these are formed during the earlier refining stages, and it is necessary to remove them in the production of a marketable white sugar. The use of ion-exchangers just before the crystallisation stage results in a significant improvement in product quality. It is necessary to use materials with an open, porous structure to allow the large colourant molecules access to the adsorption sites. Chen (1985) described the use of strongly basic resins operated in the chloride cycle for decolorisation during cane sugar refining. These are sometimes the only decolorising systems used, but in other cases complement the use of carbon adsorbents. Bohm and Schafer (1969) described the decolorisation of beet sugar juice on an industrial scale using ion-exchange resins.

A new approach to the use of ion-exchange for decolorisation of sugar solutions is the application of powdered resin technology. Finely powdered resins (0.005–0.2 mm diameter) have a very high capacity for sugar colourants due to the ready availability of adsorption sites. The use of such materials on a disposable basis eliminates the need for, and the accompanying disposal problems of, chemical regenerants, as well as removing the problem of sugar dilution which occurs during column operation. However, the advantages must be weighed against the added expense of discarding expensive resins after a single use (Chen, 1985). Colour reduction of fermentation products such as wine has also been described. Brown *et al.* (1988) used a strongly basic anion exchanger to remove colouring matter, followed by a strong cation exchanger to restore the pH. It is claimed that colour reduction can be achieved without substantially deleteriously affecting the other wine qualities.

Protein purification

Ion-exchange can be used successfully in many protein purification processes in the food and pharmaceutical industries. High purity protein isolates can be produced in a single step from dilute solutions containing other contaminating materials. The process compares favourably with competing techniques in terms of cost and efficiency. The amphoteric nature of protein molecules permits the use of either anion or cation exchangers, depending on the pH of the environment. Elution takes place by either altering the pH or increasing the ionic strength. The eluate can be a single bulk, or a series of fractions produced by stepwise or linear gradients, although fractionation may be too complex for large-scale industrial production. Separation of a single protein may take place on the basis that it has a higher affinity to the charged sites on the ion-exchanger

compared to other contaminating species, including other proteins present in the feed. In such cases, if excess quantities of the feed are used, the protein of interest can be adsorbed exclusively, despite initial adsorption of all the proteins in the feed (Kanekanian and Lewis, 1986). Alternatively it may be possible to purify a protein on the basis that it has a much lower affinity for the ion-exchanger than other proteins present in the feed, and thus the other proteins are removed, leaving the desired protein in solution.

One limitation of the process for protein treatment is that extreme conditions of pH, ionic strength and temperature must be avoided to prevent denaturation of the protein.

An area of great potential is the recovery of proteins from whey. It is estimated (van Hoogstraten, 1987) that about 110 million tonnes of whey are produced each year as a by-product of the manufacture of cheese and related products such as casein. Typically whey contains 0.6–0.8% protein, which is highly nutritious and also displays excellent physical properties, yet the vast majority of this is wasted or under-utilised. The Vistec protein recovery process employs carboxymethyl cellulosic anion-exchange materials to produce high purity functional protein from cheese whey (Jones, 1976; Palmer, 1977). The system uses a stirred tank reactor into which the whey is introduced at low pH. Following rinsing of non-adsorbed material, the protein fraction is eluted at high pH, and further purified by ultrafiltration so that the final protein content is approximately 97% (on a dry matter basis). The product is commercially exploited by the Bio-isolates company (Fig. 6.2). Ayers and Petersen (1985) have described a similar process based on sulphopropyl cellulosic materials which is also used for commercial recovery of cheese whey protein. Silica-based ion-exchangers such as Spherosil have the advantage that they are rigid and do not swell or contract when the pH or ionic strength of the environment

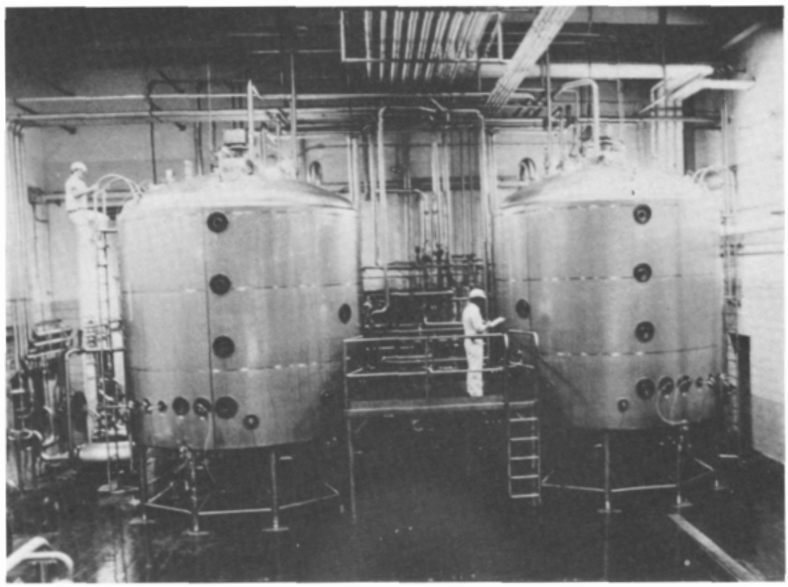

Fig. 6.2. Ion-exchange recovery of food proteins (with permission of Bio-isolates plc).

are altered, and are used commercially for whey protein recovery in the Rhône–Poulenc process (Mirabel, 1978). However, the capacity may be lower than the cellulose-based materials. Skudder (1983, 1985) has demonstrated the use of Spherosil QMA to produce fractions of total whey protein as well as further fractionating the protèins into their separate components or groups of components. This approach has the potential of producing protein fractions with a range of functional properties which could be extremely valuable for use in the food industry. The method is not yet carried out commercially to the author's knowledge, possibly due to the complex operating procedures required, and the relatively low capacity of Spherosil. Another application of adsorption of whey protein by ion-exchangers could be to improve the heat stability of milk (Kelly, 1982). The use of ion-exchange to recover or separate the caseins in milk is not carried out commercially, although it has been shown to be feasible – e.g. Ng-Kwai-Hang and Pelissier (1989).

Jones (1976) has described the use of the Vistec system (see above) for continuous recovery of food protein from protein-containing waste streams other than whey. A 10 000 gal d^{-1} pilot plant system, recovering protein from abattoir effluent, had been run continuously for 3 months. The recovered protein could then be incorporated into animal feeds. The same author has also demonstrated the use of this system for recovery of food proteins from waste streams resulting from the processing of soya, fish, vegetables and gelatine production. Such protein fractions could be used as functional proteins in the food industry.

Howell and Lawrie (1983) employed anion-exchange using DEAE-Sephadex to fractionate porcine plasma proteins in an attempt to maximise use of by-products of the meat industry. While the properties of such fractions would clearly be desirable for their use as functional food proteins, the stepwise elution may be too complex for commercial application.

Various other food proteins have been purified or fractionated by ion-exchangers, including pea globulins (Gueguen *et al.*, 1984), gliadin from wheat flour (Charbonnier and Mosse, 1980), egg protein (Parkinson, 1967) and groundnut and soya protein (Satyanarayana *et al.*, 1981).

Purification of proteins from fermentation broths usually involves a series of separation steps and frequently includes ion-exchange. Hammond and Scawen (1989) reviewed the use of ion-exchange in the high-resolution fractionation of proteins in downstream processing. Werner and Berthold (1988) discussed the purification by ion-exchange of a range of recombinant DNA-derived proteins, produced by fermentations, which can be used as active ingredients in pharmaceuticals. The industrial production of recombinant insulin from *Escherichia coli* fermentation involves an ion-exchange purification (Prouty, 1989).

Large-scale purification of a variety of enzymes has been described. For example, α-amylase recovery from *Aspergillus awamori* (Bhella and Altosaar, 1985) or L-leucine dehydrogenase from *Bacillus cereus* (Schuette *et al.*, 1985) may be achieved by ion-exchange. Porter *et al.* (1991) described the large-scale purification of β-galactosidase from soybean meal using a combination of strong anion- and strong cation exchangers.

Ion-exchange is used in the recovery, separation and purification of monoclonal antibodies which are used for high-resolution diagnostic purposes. Duffy *et al.* (1988)

describe the purification of kilogram quantities of monoclonal antibodies by ion-exchange.

Purification of other compounds

Ion-exchange has been used for numerous other separations involving food and biochemicals, which do not fit into the above categories.

Fructose production is of great interest as it is considerably sweeter than sucrose and glucose, and can be used as a natural sweetener at reduced caloric intake. Although present in many natural sources, it is produced commercially from corn starch by hydrolysis to dextrose, which is then partially converted to fructose using the enzyme isomerase. The resulting high fructose corn syrup may be deionised by ion-exchange and then a pure fructose fraction can be recovered with a sulphonic cation exchanger (such as Amberlite IR-140). The separation is based on the fact that such resins, in the divalent salt form, exhibit a slightly higher affinity for fructose than glucose (Kunin, 1979). In practice a small volume of syrup containing both sugars is placed on top of a bed of the ion-exchanger and slowly displaced with water so that the glucose travels more rapidly down the column than fructose. By alternating the feed of syrup with eluting water, a series of 'cuts' of fructose and glucose may be collected. Although the mechanism is complex, the process is carried out successfully in a number of commercial plants.

A further potential application is the production of lactose-free milk. A process using sulphonated cation exchangers has been used to reduce the lactose level of skim milk to <10% of that in the feed, while retaining >90% of protein, minerals and citrate (Harju, 1987).

Many products derived from fermentation processes are purified by ion-exchange. The following are some examples. Kunin (1974) described the recovery of the aminoglycoside antibiotics streptomycin and neomycin directly from a fermentation broth using an expanded bed which permits particulate matter to pass through without clogging the bed. Purification of fermentation-derived ethanol for use as fuel is possible using a combination of strongly basic and strongly acidic ion-exchangers (Rohm and Haas (patent), 1988). The purification of phenylalanine, which may be used in sweetener production, from fermentation broths using cationic zeolite material, has been patented (UOP (patent), 1990).

Ion-exchange may also be used to purify enzymic reaction products, e.g. Heinzler *et al.* (1987) incorporated the process in a system for recovery of flavour constituents from the enzymic degradation of fruit wastes.

6.2 ELECTRODIALYSIS

Electrodialysis (ED) can be used to separate ionic species in the food and biotechnology industries. The process permits the separation of electrolytes from non-electrolytes, concentration or depletion of electrolytes in solutions, and the exchange of ions between solutions.

6.2.1 Theory and equipment

Separation occurs due to electromigration of ions through membranes, which depends on the electrical charge on the molecules, combined with their relative permeability through membranes. Separations are based on the use of ion-selective membranes which are effectively sheets of ion-exchange resins. The membranes are composed of polymer chains which are cross-linked and intertwined into a network, and bear either fixed positive or fixed negative charges. These may be heterogeneous membranes which consist of ion-exchange resins dispersed in a polymer film, or, more commonly, homogeneous membranes in which the ionic groups ($—NH_3^+$ or $—SO_3^-$) are attached directly to the polymer. Counterions are freely exchanged by the fixed charges on the membranes and thus carry the electric current through the membranes, while co-ions are repelled by the fixed charges and cannot pass through the membrane. Therefore cation membranes allow the passage of positively charged ions, while anion membranes allow the passage of negatively charged ions.

In practice the cation and anion membranes are usually arranged alternately with plastic spacers (Fig. 6.3) to form thin solution compartments as shown schematically in Fig. 6.4. In commercial practice 100–200 membranes may be assembled to form a membrane stack (Fig. 6.5), and an ED system may be composed of one or more stacks. Commercial ED membranes may be as large as 1–2 m^2. The spacers must maintain a constant spacing between the membranes, but must not cover a large fraction of the membrane surface or cause stagnation of the fluids. This is usually achieved by either using a netting material which also promotes turbulence, or the use of a tortuous path arrangement which forces the liquid stream into a long pathway before leaving the cell. The basic unit of a membrane stack is called a cell pair and comprises a pair of membranes and spacers as illustrated in Figs. 6.3 and 6.4. A positive electrode at one end

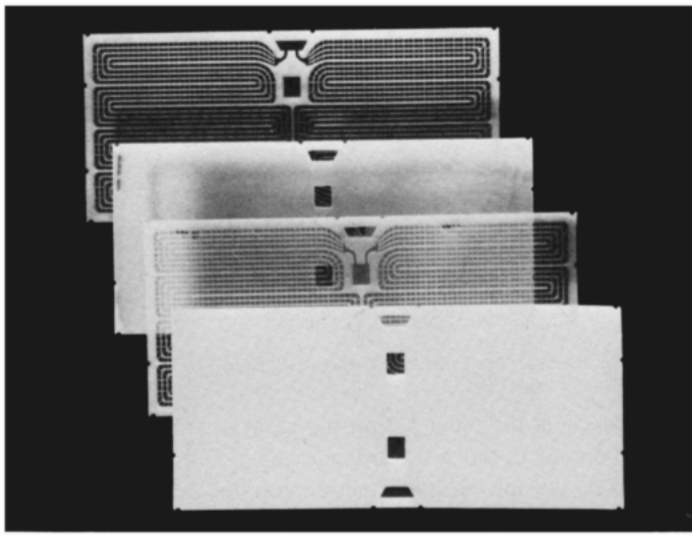

Fig. 6.3 Electrodialysis membranes and spacers (with permission of Ionics Inc.).

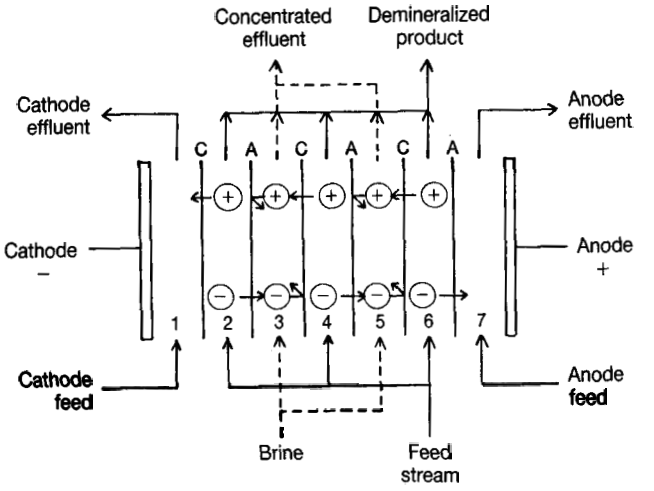

Fig. 6.4. Schematic diagram of electrodialysis process

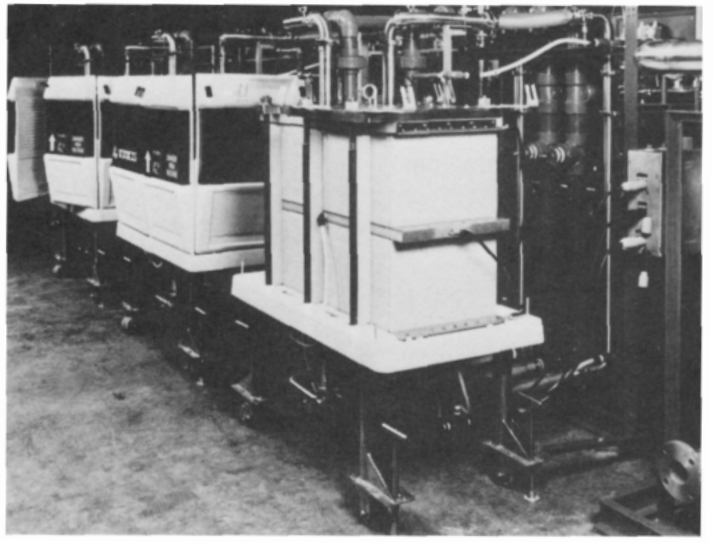

Fig. 6.5. Electrodialysis membrane stacks (with permission of Ionics Inc.).

and a negative electrode at the other permit the passage of a d.c. current. The electrical potential causes the anions to move towards the anode and the cations to move towards the cathode. However, the ion-selective membranes act as barriers to either anions or cations. Hence, anions migrating towards the anode will pass through anion membranes, but will be rejected by cation membranes, and *vice versa*. The membranes, therefore, form alternating compartments of ion-diluting (even numbered compartments in Fig. 6.4)

and ion-concentrating (odd numbered) cells. If a feed stream containing dissolved salts (e.g. cheese whey) is circulated through the ion-diluting cells and a brine solution through the concentrating cells, free mineral ions will leave the feed and be concentrated in the brine solution. Demineralisation of the feed is, therefore, achieved. Note that charged macromolecules, such as proteins, will attempt to migrate in the electrical field, but will not pass through either anion or cation membranes due to their molecular size. The efficiency of electrolyte transfer is determined by the current density and the residence time of the solutions within the membrane cells. The energy required to produce a certain separation can be calculated from Faraday's law, as described by Lopez Leiva (1988a). The electrodes are bathed in a solution of an electrolyte which is circulated to remove gases produced by the discharge of ions, and other ionised species. In practice the demineralisation is limited by the decreasing electrical conductivity of the feed as the process proceeds – e.g. 90% de-ashing is considered to be the practical limit for whey, and much greater production capacity is possible if lower levels of de-ashing are acceptable (de Boer and Robbertsen, 1983). To obtain greater levels of de-ashing it is possible to use combined ion-exchange/ED plants.

Alternative configurations of ion-exchange membranes are possible. Ion replacement can be achieved using either cation- or anion-exchange membranes only. The example shown in Fig. 6.6(a) is a process where cation-exchange membranes are used to replace X^+ ions with Y^+ ions. A more efficient substitution of ions is possible using a configuration as shown in the example for cations in Fig. 6.6(b). In this case three distinct streams are used – donor, product and acceptor. Very high degrees of substitution are possible if strong brine solutions are employed. One particular advantage of this approach is that pH adjustment can be made without increasing the salt level as would occur by addition of acid – i.e. H^+ ions are added to the solution without the addition of an anion.

Commercial applications of ED have depended on the development of membranes of high mechanical strength, low electrical resistance and high ion-selectivity. As with any membrane process ED membranes are subject to concentration polarisation and fouling. This limits the rate of demineralisation, so that the process cannot be accelerated at will by increasing the current density. In particular, the increased concentrations of salts near the membrane/brine interface may lead to the precipitation of scale. Suitable process design, especially to minimise boundary layers, and appropriate cleaning regimes must be employed. The most serious fouling problem in conventional ED is frequently fouling of the anion exchange membrane by negatively charged, colloidal organic matter. One possible solution to this is to employ 'transport depletion' in which the anion-exchange membranes are replaced by neutral membranes (Lopez Leiva, 1988a) as shown in Fig. 6.6(c). Longer processing times, easier cleaning and higher current densities may result, but effectiveness of demineralisation is reduced as only one set of membranes is selective.

6.2.2 Applications of ED in the food and biotechnology industries

The largest application of ED has been in the desalination of brackish water to produce potable water. In Japan, all the table salt consumed is produced by ED of sea water (Lopez Leiva, 1988a).

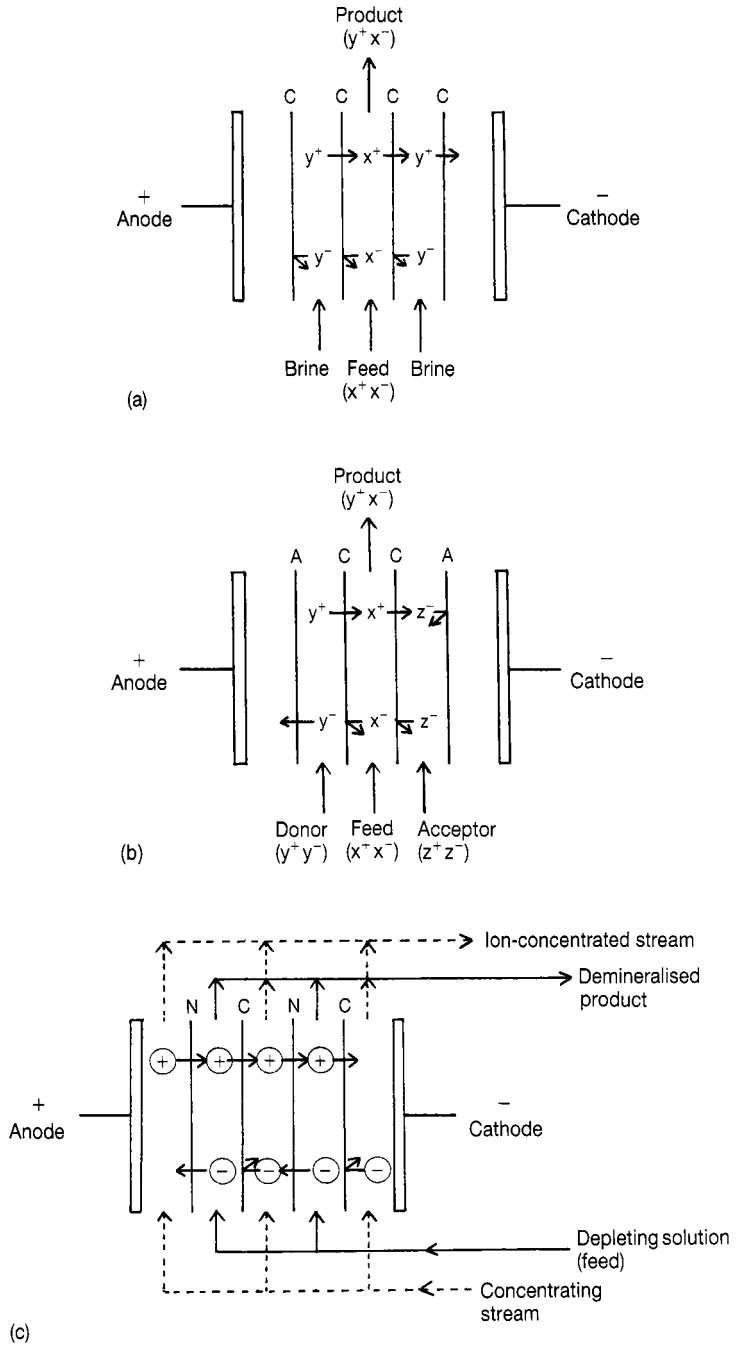

Fig. 6.6. Schematic diagrams of alternative configurations for electrodialysis: (a) ion replacement; (b) ion substitution; (c) transport depletion.

The major application of ED in the food industry is probably for desalting of cheese whey. Lopez Leiva (1988b) estimated that over 3 million tonnes of cheese whey were treated annually using over 25 000 m^2 of installed membrane area, based on annual production of the equivalent of 100 000 tonnes of 90% demineralised whey powder (Reed, 1984). Batchelder (1987) estimated a total worldwide production of 150 000 tonnes of reduced mineral solids (dry basis) per year by ED. A number of plants exist with a capacity to demineralise more than 500 000 kg per day of fluid whey. About 10% of whey solids consists of salts (largely KCl) which prohibits its direct use in infant formulae, and a high level of demineralisation is required for this application (typically 90% of the initial ash is removed from sweet whey). An estimated 65% of the ED demineralised whey finds an end use in infant formulae (Batchelder, 1987). Other uses for the reduced minerals whey include animal feeds, drinks, dry mixes, salad dressing, confectionery coatings, ice cream and bakery goods. Generally a lower level of demineralisation (26–65%) is acceptable for these other applications. In some applications the product is used as a less expensive substitute for skimmed milk powder, while in others the specific characteristics of the whey are employed – e.g. enhanced browning of baked goods. Most commercial ED plants use sweet whey which has been preconcentrated (typically about threefold) as this gives advantages with respect to the electrical conductivity of the feed. However, other feeds such as acid whey, cottage cheese whey or delactosed whey may also be processed successfully. Following ED the demineralised whey is usually concentrated further and spray dried. The production of demineralised whey is normally carried out as a batch operation as shown schematically in Fig. 6.7.

Lactic acid may be separated from whey (Coton, 1986) or soybean stock (Nomura *et al.*, 1988a) using fermentation in combination with ED. Ultrafiltration permeate may be demineralised by ED to improve lactose crystallisation (Gjersvig, 1987), but this is not carried out commercially due to the low cost of lactose.

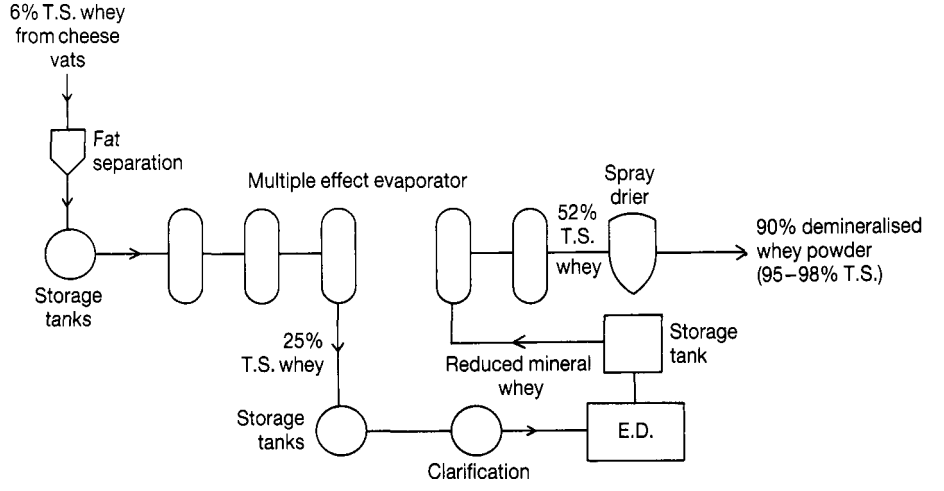

Fig. 6.7. Electrodialysis batch operation for whey demineralisation (after Batchelder, 1987).

Removal of calcium from milk has the benefit of improving the stability of the casein during freezing. Improvement of the protein stability of frozen milk following ED has been demonstrated (Lonergan *et al.*, 1982). Removal of calcium from buffalo milk by ED has also been carried out as part of a process to simulate human milk for infant nutrition (Kuchroo and Ganguli, 1980). As with ion-exchange, ED can also be used to remove radioactive metal ions from milk (Thiele, 1969).

Improved flavour and textural quality of fermented milk products following ED demineralisation and deacidification has been described (Bodor *et al.*, 1987).

ED can be used to extract salts from grape musts and wine and hence improve their stability. Relatively modest reductions in potassium are sufficient to prevent precipitate formation, the precise amounts being dependent on the type of wine (Lopez Leiva, 1988b). It is not desirable to process for longer than necessary as ED is associated with small losses of alcohol or sugar. If concentrated grape musts are used as sweetening agents in wine it is necessary to reduce levels of potassium and tartaric acid substantially in order to prevent formation of precipitates. This has been achieved successfully by ED (Audinos *et al.*, 1985; Escudier *et al.*, 1989). A further application in vinification could be to control the sugar/acid ratio in wine by either deacidification of the grape musts by ion substitution ED using anionic membranes, or acidification using cationic membranes (Wucherpfenning and Keding, 1982).

The process can be used to produce deacidified fruit juices, either to reduce the sourness of the natural juices, or possibly for the health food market. A system using only anion-exchange membranes is required, in which the citrate ions of the juice are replaced by OH^- ions. Pilot plant studies have shown that acidity of apple juice could be reduced from 1.5 to 1.1% w/v, and lemon juice from 5.8 to 4.8% w/v (Lopez Leiva, 1988b). However, Adhikary *et al.* (1987) reported that such products displayed adverse organoleptic properties.

ED could potentially be employed in the refining of sugar from either cane or beet. In fact commercial applications in these industries are limited by the severe membrane fouling problems caused by the presence of a range of organic compounds. However, a Japanese plant for the demineralisation of cane sugar syrup, following chemical pretreatment, has been described (Yamauchi *et al.*, 1985). Incorporation of ED in a process replacing carbonatation with membrane processes for the purification of sugar beet juices has been described (Hanssens *et al.*, 1984), but this has not been used commercially.

A further application of ED in the food industry is desalination of spent pickling brine (Wan Der Pan *et al.*, 1988).

ED has found a variety of applications in biotechnology. It is particularly applicable as a means of process control (e.g. altering the pH of fermentation media or enzymic reactors), or as a component of downstream processing. The process can be integrated into continuous fermentation or reactor designs.

In fermentation technology, ED has been used to isolate inhibitive metabolites to allow continuous fermentation. The possibility of increasing the production rate of lactic acid by up to six times using ED has been demonstrated (Murdia *et al.*, 1990). Similar applications include the preparation of acetic acid (Rehmann and Bansch, 1989) and propionic acid (Boyaval and Corre, 1987). Also Nomura *et al.* (1988b) have used ED for

continuous product removal, and hence increased fermentation rates, during acetic acid production. Similarly Prigent and Franco (1984) used ED for continuous extraction of sodium lactate during the fermentation of lactose. Wang *et al.* (1991) used a cation exchange membrane in the electrochemical production of L-cysteine, which has potential use in the food and pharmaceutical industries. The production of a range of amino acids using an integrated downstream process incorporating ED has also been described (Tichy *et al.*, 1990). Reed (1984) described the use of ED for the purification of genetically engineered proteins, while Aretz and Sauber (1990) have incorporated the process in the purification of enzymes. Heinzler *et al.* (1987) designed a membrane reactor incorporating ED for enzymic degradation of pectin in waste fruit products (such as apple pomace or citrus peel). The reaction products – D-galacturonic acid, oligogalacturonic acid – can be used as flavourings or pharmaceuticals.

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Chapter 7

Innovative separation methods in bioprocessing

J. A. ASENJO, Biochemical Engineering Laboratory, Department of Food Science and Technology, The University of Reading, Reading RG6 6AP, UK and **J. B. CHAUDHURI**, School of Chemical Engineering, University of Bath, Bath BA2 7AY, UK

7.1 INTRODUCTION

Discoveries and achievements in modern biology and recombinant DNA technology in the last few years have resulted in the development of a number of new therapeutics for human use such as insulin, human growth hormone (hGH), tissue plasminogen activator (tPA) for cardiac disease, erythropoietin (EPO) and hepatitis B vaccine and thus the possibility of their industrial large-scale production. This poses a tremendous challenge for the chemical and biochemical engineer in terms of developing efficient separation processes for these new proteins. As they are intended for human use the levels of purity required are of the order of 99.9% or 99.98% or even higher (depending on dosage) and they have to be separated from a very large number of contaminants, other proteins, nucleic acids, polysaccharides and many other components present in the cell culture or cell lysate used to manufacture these proteins. Competitive advantage in production depends not only on innovations in molecular biology and other areas of basic biological sciences but also on innovation and optimisation of separation and downstream processes.

The main issues important for the development of novel separation techniques to give *improved resolution, simplicity, speed, ease of scale-up* and possibly *continuous operation* are presented and discussed. The assessment of the state of the art as well as promising future developments concentrate on the separation and purification of proteins from complex mixtures. The present trend to develop techniques that exploit fundamental physicochemical principles more efficiently is emphasised. This includes the analysis of the physicochemical properties of proteins such as pI, charge as a function of pH, biological affinity (including metal ion and dye affinity), hydrophobicity and size and its

relation to efficiency in a bioseparation. Some properties (e.g. charge and affinity) can show extremely high resolution in purification operations, whereas others (e.g. molecular weight) show much lower resolution.

7.2 SYSTEM CHARACTERISTICS

7.2.1 Physicochemical basis for separation operations

Development of new and efficient separation processes will be based on more effectively exploiting differences in the actual physicochemical properties of the product such as surface charge/titration curve, surface hydrophobicity, molecular weight, biospecificity towards certain ligands (e.g. metal ions, dyes), pI and stability, compared to those of the contaminant components in the crude broth. The main physicochemical factors involved in the development of separation processes are shown in Table 7.1 (Asenjo, 1993).

Table 7.1. Physicochemical basis for the development of separation processes

Physicochemical basis	Separation process
Charge	Ion-exchange chromatography Electrodialysis Aqueous two-phase partitioning Reverse micelle extraction
Hydrophobicity	Hydrophobic interaction chromatography Reversed phase chromatography Precipitation Aqueous two-phase partitioning
Specific binding	Affinity chromatography
Size	Gel filtration Ultrafiltration Dialysis
Electric mobility	Electrophoresis
Isoelectric point	Chromatofocusing Isoelectric focusing
Sedimentation rate	Centrifugation
Surface activity	Adsorption Foam fractionation
Solubility	Solid–liquid extraction Supercritical fluid extraction

(From Asenjo, 1993)

7.2.2 Kinetics and mass transfer

The physical behaviour of the system has an effect on the development of novel separation processes. Processes can be divided into *equilibrium* and *rate* processes. In equilibrium processes selective separation depends on the attainment of a favourable equilibrium state. This, for example, includes liquid–liquid extraction and ion exchange chromatography. Rate processes, on the other hand, separate different proteins on the basis of their response to an imposed field (such as an electric field). Mobility and other similar properties determine the selectivity of this type of operation; a successful process is one in which the proteins have markedly different mobilities (e.g. electrophoresis).

In a number of protein separation processes the residence time in the reactor is insufficient for equilibrium to be achieved and the *kinetics* of adsorption play an important role for example in affinity chromatography and in the CARE (continuous adsorption recycle extraction) process. New developments in materials have recently shown dramatic advances in overcoming *mass transfer limitations* in processes such as perfusion and membrane chromatography and adsorption resulting in extremely fast separations. Some recent examples of novel techniques, which exploit the principles discussed above and provide useful analyses for optimal design of operations, include expanded bed (fluidised bed) adsorption of proteins, which allows direct broth extraction; cross-flow electrofiltration of disrupted microbial cells and for improved ultrafiltration of proteins; mathematical modelling of partitioning and phase behaviour in liquid–liquid extraction; mathematical modelling of chromatographic columns; perfusion and membrane chromatography; and advanced reversed phase chromatography using HPLC. The potential for scale-up of many of these systems is analysed and discussed.

7.3 LIQUID–LIQUID EXTRACTION: INTRODUCTION

Liquid–liquid extraction as a technology has been used in the antibiotics industry for several decades and it is now beginning to be recognised as a potentially useful separation step in protein recovery and separation, particularly because it can readily be scaled-up and can, if necessary, be operated on a continuous basis. The physicochemical factors of the protein that determine partitioning are also starting to be understood. It is a reasonably high-capacity process and can offer good selectivity for the desired protein product. However, poor solubility of the large protein molecules in typical organic solvents restricts the range of solvents available for use in such a separation process.

Two classes of solvents that appear to offer advantages for protein recovery for protein separations are aqueous polymer/salt (in some cases also polymer/polymer) systems and reverse micellar solutions. In both cases two phases are formed and the separation exploits the difference in partitioning of the proteins in the feed and extraction phases. In the aqueous polymer/salt separation systems the partitioning of the protein occurs between two immiscible aqueous phases; one rich in a polymer (usually polyethylene glycol, PEG) and the other in a salt (e.g. phosphate or sulphate). These systems show a non-denaturing solvent environment, small interfacial resistance to mass transfer, relatively high protein capacity and high selectivity. On the other hand reverse micelles exploit the solubilising properties of surfactants that can aggregate in organic solvents to form so-called inverted or reverse micelles. These aggregates consist of a polar core of

water and the solubilised protein stabilised by a surfactant shell layer. For protein extraction, one phase is the aqueous feed solution, the other the reversed micellar phase that acts as the extractant. They have several of the advantages quoted for aqueous two-phase systems.

The suitability of using foam separation as well as gas aphrons as novel separation techniques for proteins are presently under investigation.

7.3.1 Aqueous two-phase separation

Partitioning in two aqueous phases can be used for the separation of proteins from cell debris as well as for purification from other proteins. Partitioning can be done in a single step or as a multistage process. Differences in partition coefficients, however, between the different proteins can be high, hence one step tends to be sufficient (usually one for extraction and one for elution or back-extraction). The use of affinity partitioning can greatly enhance the specificity of the extraction. A typical process for extraction of a protein into the top PEG phase in a first stage and the back extraction into a bottom salt phase (e.g. phosphate or sulphate) in a second 'back extraction' step from a cell homogenate that includes recycle of the PEG phase is shown in Fig. 7.1 (Hustedt *et al.*, 1985).

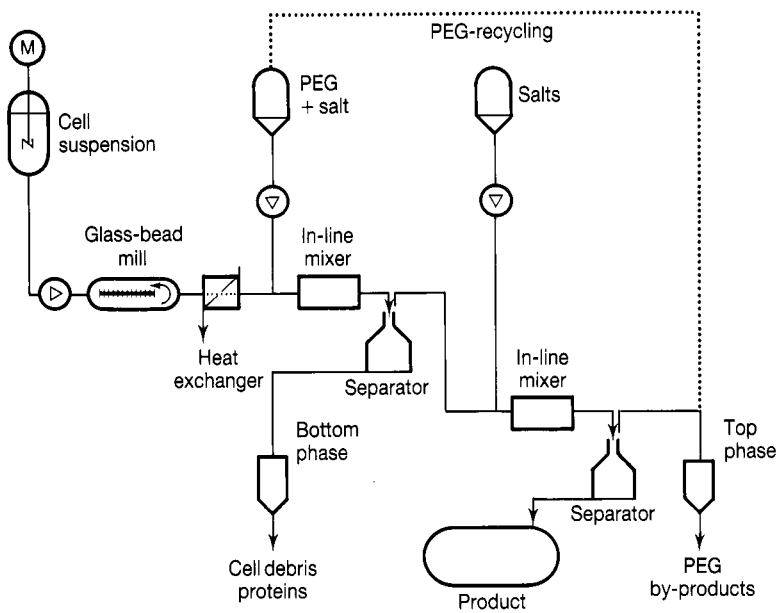

Fig. 7.1. Scheme of enzyme purification by liquid–liquid extraction. The cells are disrupted by wet milling, and after passing through a heat exchanger, PEG and salts are added into the process stream of broken cells. After mixing and obtaining of equilibrium the phase system is separated, the outflowing bottom phase is going to waste. The product-containing PEG-rich top phase goes to a second mixer after addition of more salt to the process stream. The product is recovered in the resulting bottom phase while the concentrated PEG solution (upper phase) goes to waste or is recycled. (From Hustedt *et al.*, 1985)

Most soluble and particulate material partitions to the lower, more polar (e.g. salt) phase and the protein of interest partitions to the top less polar phase, usually PEG. Separation of actual proteins in such systems is based on manipulating the partition coefficient (K) by altering parameters such as average molecular weight of the polymer, type of phase forming salt used for the heavy phase, the types of ions included in the system and ionic strength of added salts (e.g. NaCl) (Schmidt *et al.*, 1994). Figure 7.2 shows that the partition coefficient of α-amylase is a strong function of the presence of NaCl in a PEG/sulphate system. For extraction of the α-amylase from its contaminants a high concentration of NaCl is used in the first extraction stage, whereas a low concentration of NaCl in the back-extraction stage will allow the recovery of α-amylase into the bottom sulphate phase as shown in Fig. 7.1.

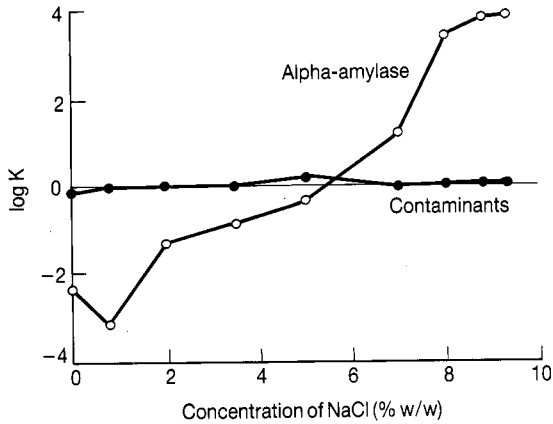

Fig. 7.2. Partition behaviour of α-amylase ($\log K_\alpha$) and contaminant protein ($\log K_c$) from industrial supernatant from *B. subtilis* fermentation in PEG 4000/Sulphate systems as a function of added NaCl concentration at pH 7 and a phase volume ratio of 1.

The partition coefficient (K) is defined as the concentration of a particular protein in the lighter phase divided by the concentration in the heavier phase. The main factors that determine partition depend on the type of system used:

(1) *Hydrophobicity.* Differences in the surface hydrophobicity between proteins are exploited when partitioning them in PEG/salt two-phase systems. Typical systems that exploit a protein's hydrophobicity are PEG/phosphate and PEG/sulphate with addition of a high concentration of NaCl (e.g. 10%).

(2) *Size-dependent partition.* Molecular size of the proteins or surface area of the particles to be partitioned is the dominating factor. It has been shown that for PEG/Dextran systems a protein's molecular weight is inversely proportional to its partition coefficient.

(3) *Electrochemical.* Electrical potential between the phases is used to separate molecules or particles according to their charge. This is demonstrated in

PEG/Dextran systems with addition of small concentration of salts whose charged ions will partition between the phases (e.g. 0.1 M NaCl or 0.05 M Na_2SO_4) and also by manipulating the pH of the system. In PEG/salt systems an increase in the pH usually increases the value of K. Figure 7.3 shows the increase in K with pH for α-amylase in a PEG/phosphate system. As the bottom phase has a high concentration of salt, it is the charges in the top phase that affect partitioning; thus the top phase must have a higher density of positive charges.

(4) *Biospecific affinity.* The affinity between sites on the proteins and ligands attached to one of the phase polymers is used for separation. Dyes, inhibitors, fatty acids, glutathione, Protein A and several other ligands have been used. Particularly impressive results for selective partitioning have been obtained with metal ions (e.g. Cu^{++}) both in PEG/Dextran but also in PEG/salt systems (Wuenschell *et al.*, 1990).

(5) *Solubility dependent.* In addition it is important to know the actual concentration of the protein in the extractant phase. In typical PEG/salt systems, protein solubility tends to be higher in the PEG and lower in the salt phase. It is clear that, in the region near saturation of the protein in one of the phases, a constant partition coefficient is no longer observed.

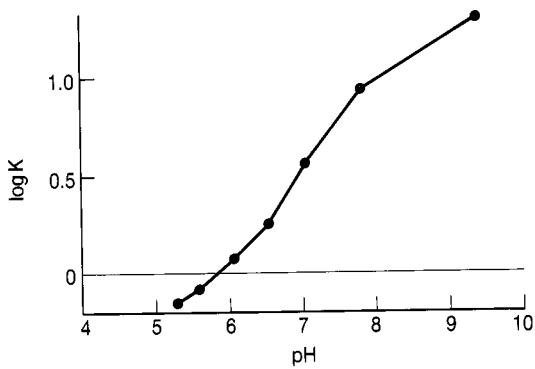

Fig. 7.3. Partition of pure α-amylase (K) in PEG 4000 (10% w/w)/phosphate (11.5% w/w) systems as a function of pH.

According to this, it is possible to split the partition coefficient into different terms:

$$K = K_{hfob}K_{el}K_{mw}K_{aff}K_{sol}$$

where hfob, el, mw, aff and sol stand for hydrophobicity, electrostatic, size (molecular weight), affinity and solubility contributions to the partition coefficient.

In practical terms partitioning in aqueous two-phase systems is influenced by many system variables. Generally, the higher the molecular weight of the polymers the lower the concentration needed for the formation of two phases. Also, the larger the molecular weight of the PEG, the lower the value of K. Work is presently being carried out on elucidating how the different physicochemical properties of individual proteins determine

their partition behaviour in two phase systems (Hachem, 1992; Asenjo *et al.*, 1994; Schmidt, 1994).

It has been possible to correlate the partition coefficient of a representative number of proteins to their hydrophobicity measured by precipitation. This correlation was not as good if the hydrophobicity was evaluated by hydrophobic interaction chromatography (HIC) or also by reverse phase-HPLC (RP-HPLC) (Hachem, 1992; Asenjo *et al.*, 1994; Hachem *et al.*, 1994). In a typical protein precipitation graph as that shown in Fig. 7.4 (S = protein in solution), the protein's solubility (and thus 'hydrophilicity') can be expressed by point m^*, which is the point at which the protein starts precipitating. Thus the hydrophobicity, P, was evaluated as $1/m^*$. The correlation found between hydrophobicity and partition in PEG/salt systems with a high concentration of NaCl is shown in Fig. 7.5. This can be represented by the equation

$$\log K = D\,\Delta W\ \log P - D\,\Delta W\ \log P_0$$

ΔW corresponds to the tie-line length of a system and is evaluated by the difference in concentration of one component (e.g. PEG or salt) between the phases which is constant for one particular system. D is the 'discrimination factor' and thus $D\,\Delta W$ is the slope in Fig. 7.5 which corresponds to the resolution of a particular system to exploit differences in hydrophobicity between proteins. Table 7.2 gives values of resolution ($D\,\Delta W$) and 'intrinsic hydrophobicity' (P_0) found for PEG/phosphate systems with different concentrations of NaCl. Clearly the systems with higher concentrations of NaCl give a higher resolution to exploit the protein's hydrophobicity in partitioning.

7.3.2 Reverse micelle extraction

Water-in-oil microemulsions, or reverse micelles, are stable, monodisperse aggregates of surface-active molecules (1–10 nm diameter) in an organic solvent. Typically, the

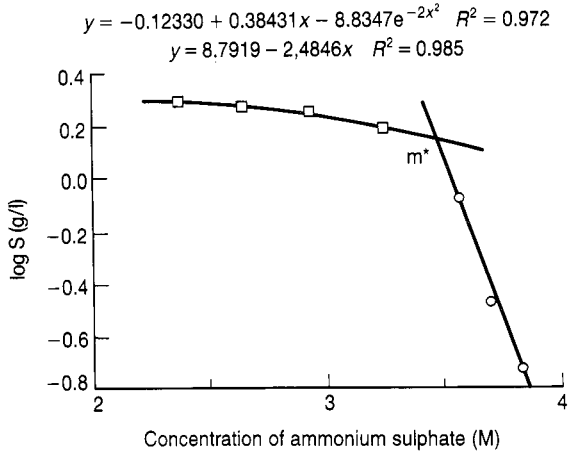

Fig. 7.4. The fitted curves of the solubility data of β-lactoglobulin A at 25°C in a solution containing added ammonium sulphate. The equation at the top represents the salting-in region and the equation below the first represents the salting-out region.

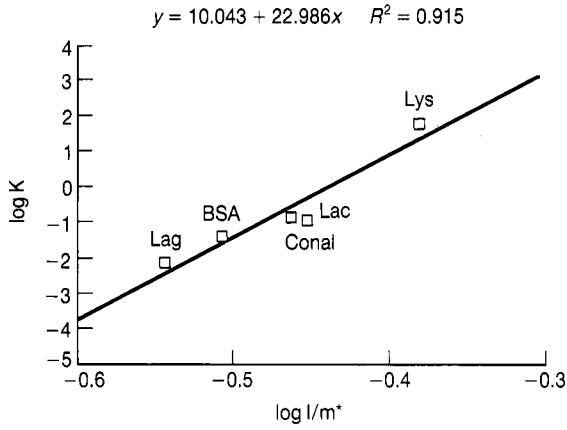

Fig. 7.5. The relationship between log K of the model proteins partitioned in two-phase system made of 8% PEG and 12% PO_4^{3-} (pH 7.0) to which 9.6% (w/w) NaCl was added and their log $(1/m^*)$. Experiments were carried out at room temperature. Abbreviations are lysozyme (Lys), α-lactalbumin (Lac), β-lactoglobulin (Lag) A, conalbumin (Conal), and bovine serum albumin (BSA).

Table 7.2. The calculated values of $D\,\Delta W$ and the intrinsic hydrophobicity, log P_0, of the aqueous two-phase systems used at 20°C

Two-phase system (wt %)	$D\,\Delta W$	log P_0
8% PEG, 12% PO_4^{3-}	5.4	–0.23
8% PEG, 12% PO_4^{3-} + 0.48% NaCl	8.3	–0.29
8% PEG, 12% PO_4^{3-} + 4.8% NaCl	14.9	–0.38
8% PEG, 12% PO_4^{3-} + 9.6% NaCl	22.4	–0.45
8% PEG, 12% PO_4^{3-} + 17.6% NaCl	22.8	–0.45

surfactant Aerosol-OT (AOT) in isooctane is used because AOT can solubilise a large amount of water in isooctane and similar hydrocarbons, thus forming reverse micelles, without the use of co-surfactants. Figure 7.6 shows a diagram of a protein partitioning into reverse micelles. Water pools exist within these microemulsions, and are stabilised by the surfactant. This system can be manipulated so that certain protein species will partition into and out of the water pools, which are a suitable environment in which proteins may exist shielded from the denaturing organic phase (Luisi *et al.*, 1988). Thus, reverse micelle solutions have the potential to be used as an extractant phase in a separation process for proteins, offering the following advantages of conventional liquid–liquid extraction: already established continuous processes; use of inexpensive solvents; and high volumetric capacities, but with greater selectivity than solvent extraction.

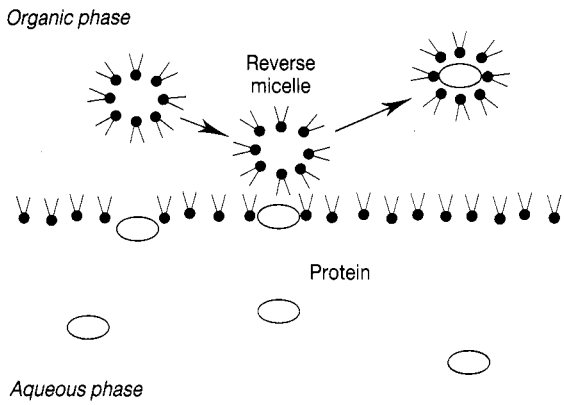

Fig. 7.6. Protein partition into reverse micelles.

There are two techniques for transferring proteins into the micellar phase. The most widely used method involves extraction of the protein with a biphasic liquid system, i.e. liquid–liquid extraction. One phase is the aqueous solution of the protein, and the other the organic micellar solution, usually in equal volume. By gently shaking the two phases, the protein partitions from the aqueous into the micellar phase. In the second method, solid state extraction of the protein, the protein powder is suspended in the micellar phase and gently stirred.

The protein solubilised in the reverse micellar solution can be transferred back into an aqueous solution, by contacting the micellar solution with an aqueous solution containing a high concentration of a particular salt (KCl, $CaCl_2$), which has the capability to exchange with the protein in the micelles.

The basic idea is that the process of protein extraction by reverse micelles can be made specific (i.e. tailored to a specific protein) and efficient (i.e. high extraction yield) by controlling the micellar parameters such as the water content, the type and concentration of surfactant, the type and concentration of salt, and the pH.

Leser *et al.* (1986) examined the transfer of ribonuclease-A, lysozyme, trypsin and pepsin, monitoring the protein concentration and the concentration of water found in the organic phase. It was observed that the transfer of water is generally moderate (below 4%), whereas, under certain conditions, the protein is quantitatively transferred. This fact demonstrated that the transfer of the protein into the micellar phase is not a passive process, i.e. is not simply due to the fact that water is transferred and with it the protein.

The conclusion was that there is a thermodynamic driving force for the hydrophilic protein to leave the aqueous environment and to transfer into the reverse micelles. In other words, it seems that under certain conditions the protein-reverse micelle complex is energetically favoured above the free protein and empty reverse micelles. Interactions can be electrostatic, when surfactants with charged head groups are used, or hydrophobic with the surfactant interface or the apolar solvent.

The fact that electrostatic interactions play an important role in the distribution of proteins over reverse micellar and aqueous phase is shown by the dependence of the aqueous phase pH and ionic strength.

The pH of the solution will affect the solubilisation characteristics of a protein primarily in the way in which it modifies the charge distribution over the protein surface. With increasing pH the protein becomes less positively charged until it reaches its isoelectric point (pI). At pHs above the pI the protein will take on a net negative charge. If electrostatic interactions play a significant role in the solubilisation process, partition with anionic surfactants should be possible only at pHs below the pI of the protein, where the protein is positively charged and electrostatic attractions between the protein and the surfactant head groups are favourable. At pHs above the pI, electrostatic repulsions would inhibit protein solubilisation.

Göklen and Hatton (1987) have presented results on the effect of pH on solubilisation of cytochrome-c, lysozyme, and ribonuclease-A, in AOT/isooctane reverse micelle solutions. The results were presented as the percentage of the protein transferred from a 1 mg/ml aqueous protein solution to an equal volume of isooctane containing 50 mM of the anionic surfactant AOT. A summary of their results is presented in Table 7.3.

Table 7.3. Effect of pH on solubilisation

Protein	pI	pH range of maximum solubilisation
cytochrome-c	10.6	5–10
ribonuclease-A	7.8	1–7
lysozyme	11.1	6–11

As anticipated, only at pHs lower than the pI was there any appreciable solubilisation of a given protein, while above the pI the solubilisation appears to be totally suppressed. However, at extremes of pH there is a drop in the degree of solubilisation of the proteins due to protein denaturation, observed as precipitate formation at the interface (Chaudhuri *et al.*, 1993).

Luisi *et al.* (1979) used the quaternary ammonium salt methyl-trioctylammonium chloride (TOMAC) for the transfer of α-chymotrypsin from water to cyclohexane. It was found that the pH had to be reduced to values significantly below the pI (pI = 8.3) for there to be any appreciable solubilisation. The solubilisation occurred only over a very narrow pH range before decreasing rapidly again with further decreases in the pH of the aqueous feed phase, accompanied by precipitation at the interface.

Similar results have been obtained by Dekker *et al.* (1986) for the enzyme α-amylase. Significant solubilisation of the enzyme was observed over a narrow pH range in the vicinity of 10–10.5 (pI = 5.1). In this pH range, all basic residues will be deprotonated and the only charged residues being the carboxyl groups bearing a negative charge.

These results suggest that for higher molecular weight proteins a precise match between the surface charge densities of the protein and the reverse micelle is needed for the transfer to occur.

The effect of the ionic strength of the aqueous phase is primarily to mediate the electrostatic interactions between the protein surface and the surfactant headgroups. As a result of Debye screening, increases in the ionic strength of the protein feed solution can be expected to reduce the interaction between the protein and surfactant headgroups, hence decreasing the solubilisation of the protein. This has been studied by Göklen and Hatton (1987). The transfer to the reverse micellar phase decreases for all proteins studied (cytochrome-c, lysozyme and ribonuclease-A) at increasing ionic strength, but the point where this decrease starts depends on each particular protein. Such studies have shown that protein transfer is influenced by electrostatic interactions between the protein and surfactant headgroup, and that selectivity with respect to other proteins can be controlled by manipulation of pH and ionic strength (Göklen and Hatton, 1987).

Other recent fundamental research in this field has focused on aspects of the kinetics of protein partition and the structure of the microemulsions (Fletcher *et al.*, 1987; Luisi *et al.*, 1988). Other work has indicated the considerable potential for the use of reverse micelles in selective protein purification (Dekker *et al.*, 1989). Woll *et al.* (1989) have shown that affinity ligands can be accommodated within the micelles to enhance selectivity.

A study on the fractionation of intracellular proteins from Bakers' yeast (Chaudhuri, 1991) proved to be complex, with problems of low protein yield, interfacial precipitation and denaturation, which were not evident in the studies with model solutions. As a result of disrupting the yeast cells, the intracellular contents (sugars, lipids, nucleic acid etc.) will be present as 'impurities'. It is not known if these non-protein components contributed to the observed precipitation and denaturation, or if they can partition into the microemulsion, thereby reducing protein capacity and selectivity.

There has been some activity in this field: for example, Schomaecker *et al.* (1988) have observed the inactivation of α-chymotrypsin in an AOT/*n*-heptane system. However, they concluded that activity was lost as a result of enzyme autolysis as well as by interaction with the microemulsion system.

Finally, the use of reverse micelles to isolate and refold pure denatured proteins has been studied (Hagen *et al.*, 1990a) as shown in Fig. 7.7. The results reported 100% renaturation of denatured ribonuclease-A refolded in the reverse micelles. This method proved unsuitable for the refolding of interferon-γ because its hydrophobic nature causes it to aggregate during the extraction process (Hagen *et al.*, 1990b). There remain several unanswered questions regarding the use of reverse micelles, for example, how applicable is this method to other proteins, how will protein from inclusion bodies behave in this system, and is any activity lost during the extraction process?

7.3.3 Perfluorocarbon affinity separations

Affinity chromatography exploits the natural, biospecific interactions that occur between biological molecules. These interactions are very specific and because of this affinity separation processes are very high resolution methods for the purification of proteins. However, conventional affinity chromatography has its drawbacks. Namely the gel

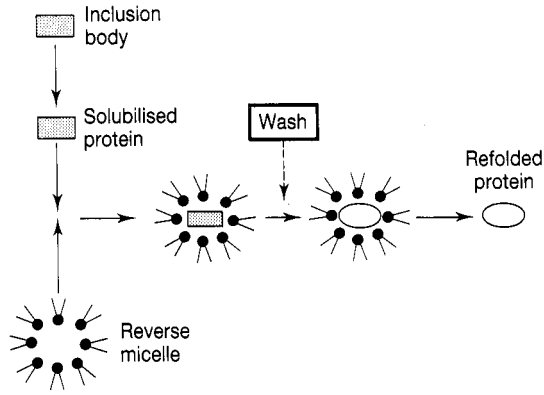

Fig. 7.7. Schematic diagram of protein refolding in reverse micelles.

matrix used as the support for the affinity ligand is not very stable towards extremes of pH which are found during sterilisation, and fouling of the columns may occur unless all particulate material is removed prior to application on the column. This requires the use of a solid–liquid separation operation increasing the process costs.

A development in affinity purification is the use of perfluorocarbons as supports for affinity chromatography. Perfluorocarbons are synthetic molecules consisting of only carbon and fluorine. These compounds are chemically and biologically inert and are insoluble in both organic and aqueous solutions. However, the extreme hydrophobicity of perfluorocarbons would lead to protein denaturation on contact. The perfluorocarbon can be wetted in the presence of fluorosurfactants which adsorb to the perfluorocarbon. By attaching a triazine dye molecule to the fluorosurfactant an affinity ligand is constructed. The perfluorocarbon emulsion is formed by homogenising the perfluorocarbon in the presence of the surfactant. The resulting emulsion droplets have diameters ranging from 10 to 37 μm (McCreath *et al.*, 1992). The droplet size may be manipulated through the emulsification conditions. Following emulsification the surfactant is cross-linked using glutaraldehyde in the presence of HCl. The reduction of ligand leakage may be achieved by derivatising poly(vinyl alcohol) PVA with fluoroalkyl groups and then using this to coat the perfluorocarbon surface.

Perfluorocarbon emulsions have been utilised in an expanded bed configuration. The perfluorocarbon emulsion must be used in an expanded bed to get round the problems of droplet compression which would occur in a fixed bed column. The additional advantage of this method is that the expanded bed arrangement allows any solid particles to flow around the suspended emulsion droplets, thereby giving the potential for direct product removal from fermentation or cell culture broths. The emulsion droplets are allowed to settle in a column and are fluidised by the upward flow of buffer through the bed. At the maximum flow rate this resulted in an expanded bed twice the height of the settled bed (McCreath *et al.*, 1992). By the use of the ligand CI Reactive Blue 4 such a system has been used to adsorb human serum albumin (HSA) from plasma (McCreath *et al.*, 1992). The HSA was recovered at 87% yield and 91% purity. The purification factor was 1.44.

More recently this technique has been exploited in a continuous reactor for protein purification. Current chromatographic practice results in batch protein purification with the product being eluted and recovered at one stage in the process. Continuous separation would enable the protein to be recovered continuously, and would be amenable to scale up more easily than conventional chromatography. The emulsion reactor is based on liquid–liquid contact between the perfluorocarbon emulsion and the protein solution. The high density of the perfluorocarbon (1.8–2.1 g/ml, Stewart *et al.*, 1992) results in fast settling of the emulsion and aqueous phases and thus is suitable for a liquid–liquid extraction process. The protein is adsorbed onto the affinity perfluorocarbon emulsion which separates from the depleted aqueous phase under gravity. The loaded perfluorocarbon emulsion is eluted with a buffer to recover the protein and then re-equilibrated for further use. Continuous protein purification is carried out using a four-chambered mixer–settler type configuration known as a perfluorocarbon emulsion reactor for continuous affinity separations (PERCAS) (McCreath *et al.*, 1993). Each chamber is identical and consists of a mixing zone agitated by a turbine. The perfluorocarbon–aqueous mixture passes over a weir and into a settling chamber, where the perfluorocarbon emulsion settles to the bottom with the aqueous phase on top. Either phase can then be pumped out either into the top of the next chamber or to waste. In the first chamber the adsorption of the protein onto the emulsion takes place. After settling the loaded emulsion is pumped into the second mixing chamber, where it is washed to remove any trapped contaminants. The washed emulsion is pumped into the third chamber, where the protein is eluted and subsequently recovered. The depleted emulsion is pumped into a fourth chamber, where it is washed and re-equilibrated, and then pumped into the first chamber to adsorb more protein.

This process was tested using the adsorption of HSA from plasma using the dye ligand CI Reactive Blue 2 attached to the perfluorocarbon emulsion described above (McCreath *et al.*, 1993). Protein binding was found to fit the Langmuir isotherm. The continuous protein separation was controlled by varying the aqueous flow rates; the emulsion flow rate was kept constant. The total protein recovery was 89% with HSA recovery at 81%. The overall process yield was 71% with the HSA recovered at 91% purity (purification factor of 1.52).

The perfluorocarbon emulsions behave as normal chromatographic materials when operated in a fluidised bed. The protein adsorption capacities are comparable to conventional matrices. Advantages of these materials are the fast adsorption and desorption which arise as the droplets are non-porous. The advantages of the PERCAS system are the continuous nature of the operation and the relative simplicity of the system – a mixer–settler combined with gravity settling.

7.3.4 Liquid membrane separations

Liquid membrane extraction is a relatively new separation technology which has significant potential for the selective separation and concentration of low molecular weight chemicals produced by fermentation and used in the food-processing industries. Separation is achieved by the transport of the solute from a feed phase across a film of organic solvent into a stripping phase. Examples of products successfully extracted using liquid membranes include organic acids such as citric acid (Boey *et al.*, 1987), lactic acid

(Chaudhuri and Pyle, 1990), and amino acids such as L-phenylalanine (Itoh *et al.*, 1990). Currently, the recovery and purification processes for these species involve several steps specific to individual manufacturers. There is, therefore, scope for the application of liquid membranes which are generally one-step processes, and can simultaneously separate and concentrate the solute. A schematic diagram of a liquid membrane process is shown in Fig. 7.8.

The liquid membrane consists of the organic solvent which separates the two aqueous phases (the feed and stripping phases), and contains a carrier species to enhance both selectivity and rates of extraction. Aliphatic diluents are generally preferred as the solvent because of their lower solubility in water. In an ideal situation the solvent should have no solubility in water to ensure that there is no aqueous phase contamination by trace organics. There are two main configurations by which liquid membrane extraction can be exploited, as discussed in the next two paragraphs.

A supported liquid membrane (SLM, Fig. 7.9) can be achieved by impregnating a porous solid film with an organic solvent, which is held in place by capillary forces that exist within the pores. In order for the membrane pores to be effectively wetted, the surface tension of the solvent must be less than the critical surface tension of the membrane polymer. The membrane separates an aqueous phase, initially containing the required species, from an aqueous phase into which the solute is extracted, the stripping phase. The solid supports used are generally microporous polymeric films, e.g.

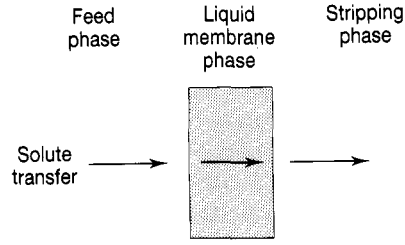

Fig. 7.8. Schematic diagram of liquid membrane processes.

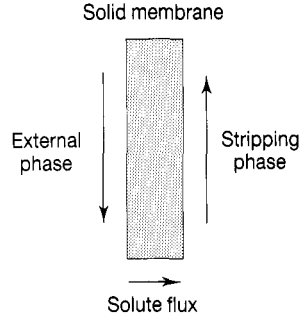

Fig. 7.9. Schematic diagram of supported liquid membrane (SLM).

polypropylene, polysulphone, or other hydrophobic materials. Typical dimensions are a membrane thickness of 25–50 μm, with pore sizes of 0.02–1.0 μm.

An emulsion liquid membrane (ELM, Fig. 7.10) is formed by creating, under high shear, a dispersion of the stripping phase within the organic solvent which forms a non-porous film around the stripping phase droplets. The emulsion thus formed (stabilised by a surfactant) is dispersed into the feed phase containing the solute, which is then transported into the stripping phase. Depending on the dispersion conditions the globule diameter is 1–2 mm and the internal phase droplets are micron sized. The two aqueous phases cannot physically contact each other and the solute is transported into the internal phase droplets by diffusion through the stabilised solvent film. The use of a chemical reagent in the stripping phase, which reacts with the extracted solute, prevents the solute from diffusing back across the membrane phase. This strategy allows the removal of virtually all of the solute from the feed solution, which makes emulsion liquid membrane extraction very attractive for the recovery of solutes formed in low concentration. After the extraction step the solute is recovered by allowing the emulsion and feed phases to separate, by settling under gravity, then removing the emulsion and breaking it to release the separated solute from the membrane phase components. Electrostatic splitting is generally used for de-emulsification as the membrane components can be recycled for further use.

ELM systems give rise to very fast extraction kinetics and allow the use of conventional liquid–liquid extraction equipment; they are also prone to emulsion swelling, which gives rise to dilution and instability problems. The necessity to make and break an emulsion does not arise with the SLM system; however, this configuration has slower kinetics, and loss of the membrane phase may occur. In summary, liquid membrane processes offer high separation factors, low capital and operating costs, a lower solvent inventory than solvent extraction, ease of scale-up and the possibility of continuous operation.

The key to selectivity in liquid membrane extraction is the use of a carrier species incorporated in the organic solvent to increase the solute solubility: by introducing a

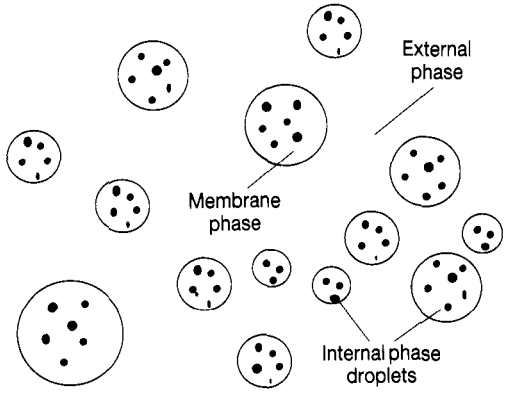

Fig. 7.10. Schematic diagram of emulsion liquid membrane (ELM).

'carrier' molecule into the membrane phase, the solute solubility is increased by the reversible formation of a membrane-soluble carrier–solute complex. This results in faster mass transfer rates, and selectivity is introduced into the extraction as the carrier–solute reaction can be selective. This is known as facilitated transport. The use of a carrier enhances selectivity by the formation of a reversible complex between the carrier and the solute, which is only soluble in the organic solvent. This is particularly effective for the recovery of charged solutes which may be poorly soluble in the organic solvent. Many of the carriers so far employed in liquid membrane processes are extractants used in conventional liquid–liquid extraction, e.g. secondary and tertiary amines, and phosphorus-containing extractants.

There have been several applications of liquid membrane extraction to biotechnological separations. The recovery of citric acid has been studied by Boey *et al.* (1987). The liquid membrane consisted of Alamine 336 and Span 80 dissolved in Shellsol A. Sodium carbonate was used as the internal phase reagent. This work looked at the batch extraction of both model and real fermentation broths. The results show that very fast extraction of citric acid can be achieved: over 80% of a 5% (w/v) citric acid solution was removed in under 5 min. Significant emulsion swelling was also observed in this study; the volume of the internal phase was more than doubled. The recovery of citric acid in supported liquid membranes has also been reported (Sirman *et al.*, 1990).

Lactic acid extraction has also been achieved using emulsion liquid membranes (Chaudhuri and Pyle, 1992a, b). The tertiary amine Alamine 336 was used as the carrier species. Extraction yields were found to be low when recovery was attempted from real fermentation broth. This was a result of competition for the carrier by 'impurities' in the broth. In this study significant swelling was observed; however, it was also possible to eliminate the effects of swelling by adjustment of the initial osmotic pressure difference.

Schöller *et al.* (1993) have found that recovery of lactic acid from homogeneous 'model' solutions resulted in fast extraction with significant product concentration, whilst extractions attempted from *Lactobacillus delbreuckii* fermentation broth exhibited poor extraction kinetics and yields. This was believed to be a result of low carrier selectivity for the solute of interest and competition by other compounds present.

Work on the extraction of phenylalanine using emulsion systems has been carried out (Itoh *et al.*, 1990). In common with other studies, work has focused on the recovery of the solute from a 'model' fermentation broth. In this study the carrier used was Aliquat 336 (tricaprylmethylammonium chloride), in Solvent 100N, stabilised by the surfactant Paranox 100. In common with other work, significant emulsion swelling was observed. It was found that swelling increased with the concentration of the internal phase reagent.

The nucleotides adenosine and deoxyadenosine have been transported through a chloroform liquid membrane using lipophilic carriers. Adenosine mono- and diphosphate (AMP and ADP) have also been transported through chloroform using a lipophilic diammonium salt of diazobicyclooctane (Pellegrino and Noble, 1990).

Chiral separation, or the resolution of optical isomers has been attempted for the transport of amino acids with 4–28% enantioselectivity. Chiral transport of sodium mandelate has been used on the optically active carrier *N*-(1-napthyl)methyl- 7α l-methylbenzylamine dissolved in chloroform. The choice of anion was found to influence chiral selectivity. Separation of racemic *N*-(3,5-dinitrobenzoyl) 7α l-amino acid

derivatives was achieved using (S)-*N*-(1-napthyl)-leucine octadecyl ester as the carrier in dodecane (Pellegrino and Noble, 1990). Racemic amine salts have been separated into their optical isomers by complexation with an optically active macrocyclic ether and transported from one aqueous phase, through chloroform, into a second aqueous phase.

The application of liquid membranes as a separation technique has mainly been in the field of hydrometallurgy, but more recently in biotechnology. Some of these applications have been described above. It is likely that more applications will become apparent through advances in carrier chemistry.

7.4 SOLID-BASED SEPARATIONS

Various new and unconventional technologies for biological molecules are presently being developed. These include systems that facilitate the handling of materials by the use of membrane, fluidised bed and novel chromatographic matrix technologies, improving separation specificity and efficiency by using metal, dye and other ligands and also by developing techniques used very efficiently at the analytical scale such as isoelectric focusing now at preparative scale.

7.4.1 Adsorption systems: expanded bed adsorption

Expanded bed adsorption or fluidised bed adsorption is a new technique that in one step accomplishes removal of whole cells and cell debris, concentration and initial purification of the target protein. It is based on the design of an adsorbent whose density enables the formation of a stable expanded or fluidised bed (two- or threefold expansion) that allows the establishment of a plug-flow concentration profile across the column. Binding capacities for proteins are similar to those obtained in fixed bed adsorption and linear velocities can be higher than 300 cm/h (Janson and Arve, 1993). Pharmacia has recently started to commercialise expanded bed adsorption under the trade name Streamline. This operation allows the fusing of two or three operations, namely clarification, concentration and capture, into one operation. At the outset, the adsorbent is sedimented in the column. The bed is then expanded by pumping buffer upward through the column. Once the bed is expanded, the buffer is replaced with crude feed which contains the products, cells, cell debris, contaminants and other particulates. After the product is adsorbed, loosely bound material is flushed with a buffer wash and the direction of the liquid flow is reversed with the change to elution buffer and the product is eluted as in packed bed chromatography. If the product is extracellular the process stream is the crude fermentation broth and if it is intracellular the stream is the crude homogenate.

The adsorption behaviour of a fluidised bed has been investigated in detail (Draeger and Chase, 1990). A frontal analysis during adsorption to investigate the shape of the breakthrough curve is shown in Fig. 7.11. The shapes of the two curves for the adsorption of BSA onto Q-Sepharose FF are very similar, suggesting that the fluidised bed system is behaving in much the same way as the fixed bed system. The apparent maximum adsorption capacity calculated from both runs was 80 g/l, which is consistent with the values from the batch isotherm experiments. A theoretical model for predicting adsorption performance in a fixed bed was used to fit theoretical curves to the data from both a fluidised or expanded bed and fixed bed systems (Draeger and Chase, 1990). The model

predicted the performance of the system very accurately, indicating again the similarity between the fluidised and fixed bed systems.

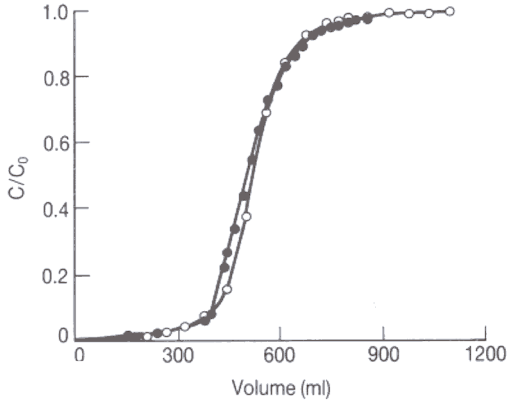

Fig. 7.11. Fixed and fluidised bed adsorption of BSA to Q-Sepharose fast flow in 0.01 M Tris buffer pH 7.0 (• fixed bed, o fluidised bed).

7.4.2 Continuous adsorption recycle extraction

Continuous adsorption recycle extraction was recently adapted for biotechnological applications as a downstream process for efficient separation of proteins from crude feedstocks to be carried out continuously (Pungor *et al.*, 1987; Gordon *et al.*, 1990). Modern biotechnology involves relatively small-scale processes that favour batch procedures, usually following the concept that a batch is well defined (from beginning to end) and that this will pose fewer problems with regulatory approval for therapeutic use. However, it is well known that in the chemical and biochemical process industries batch-to-batch variations can be substantial. It is also well known that a continuous process can operate under steady-state conditions minimising batch-to-batch variations. In addition, a continuous process is much more appropriate for implementing a control strategy in order to maintain constant conditions more readily (Rodrigues *et al.*, 1992).

Continuous adsorption recycle extraction, also called CARE, is based on the continuous protein adsorption to solid phase supports and features two well-mixed reactors with solids recycle, as shown in Fig. 7.12. The adsorbing stage takes place in the first reactor (adsorber) where the liquid feed is contacted with the adsorbent; desorption of the protein to be purified is obtained in the second reactor (desorber) by maintaining appropriate conditions and by the action of a suitable eluting solvent. The adsorbent beads are recycled to the adsorber reactor while the product is continuously removed. The system's performance is determined by the nature of the adsorbent and the feed material, flow rates and reactor volume and the conditions of the adsorption and desorption stages. An accurate mathematical model has recently been developed which can be used for investigating optimisation criteria, but also is important for system design and process control (Rodrigues *et al.*, 1992).

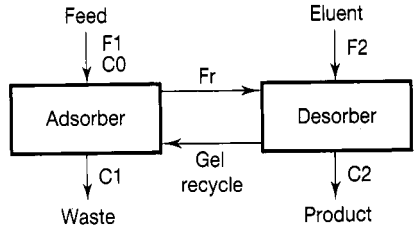

Fig. 7.12. Continuous adsorption recycle extraction (CARE) process (see text for key to symbols).

During operation, the feed composition and flow rate may experience uncontrolled variations which may upset the steady state. This is also true for adsorbent binding capacity and even recycle flow rates and composition. Hence, appropriate control schemes are needed to meet product specifications and achieve operational stability in the face of potential external and internal disturbances.

As a first approximation, the two reactors (adsorber and desorber) are assumed to be perfectly mixed (Pungor *et al.*, 1987). The adsorption process is regarded as a reversible second-order reaction, whereas the desorption stage is modelled as a first-order irreversible reaction scheme (Rodrigues *et al.*, 1992):

$$\text{adsorption:} \quad A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AB$$

$$\text{desorption:} \quad AB \xrightarrow{k_3} A + B$$

where A, B and AB are the target protein, the adsorbent and the adsorbed protein, respectively. The rate constants, k_{1-3}, represent not only the intrinsic adsorption and desorption kinetics, but also include contributions from both external and internal mass transfer resistances (Sherwood *et al.*, 1975). Thus, these effects are lumped into a single coefficient which can be determined experimentally (Chase, 1984). This approach makes the mathematical formulation more tractable at the expense of a less rigorous physical description. On the other hand, experimental evidence suggests that in such a system external mass transfer limitations can be neglected without loss of accuracy (Pungor *et al.*, 1987) and, in the case of high molecular weight proteins, these are mostly adsorbed in active sites located at or near the gel's surface since molecules attached to those sites block the way to diffusion into the gel. Any thermal effect associated with adsorption and desorption is neglected and the operation is carried out under isothermal conditions. The ordinary differential equations system can be easily solved using the Runge–Kutta fourth-order algorithm. The steady-state solution is obtained by setting the accumulation terms (i.e. time derivatives) equal to zero, giving rise to a set of non-linear algebraic equations which can be solved analytically. The steady-state model provides a valuable tool in the formulation of control schemes.

Table 7.4 shows the parametric sensitivity of steady state outputs, when each input varies 20% around the baseline value. The sensitivity index S was calculated as the ratio between the percentage variation of the output and the percentage variation of the input,

Table 7.4. Steady state sensitivity analysis (sensitivity index, S)

	Output				
Input	C_1	C_2	q_1	q_2	C_2/C_0
C_0	1.2	0.4	0.4	0.4	0.4
F_1	0.7	0.3	0.3	0.3	0.2
F_2	–0.1	–1.0	–0.1	–0.1	–0.6
F_R	–0.6	0.5	–0.3	0.7	0.4
q_m	–0.7	0.6	0.6	0.6	0.1
k_1	–0.3	0.2	0.2	0.2	0.2
k_2	0.3	–0.2	–0.2	–0.2	–0.2
k_3	0.0	0.0	0.0	0.8	0.0

q_1 = bound protein concentration in adsorber
q_2 = bound protein concentration in desorber
q_m = maximum adsorption capacity of gel
All other variables and parameters are defined in Fig. 7.13 and in text.

and represents the corresponding steady state gain. It can be seen that all outputs are strongly affected by the feed protein concentration (C_0), the gel recycle rate (F_R), the gel maximum adsorption capacity (q_m) and, to a lesser extent, by the feed flowrate (F_1). The eluent flowrate (F_2) has a strong influence on the product stream concentration (C_2) and the protein recovery ratio (C_2/C_0). The kinetic parameters (k_{1-3}) do not seem to have a strong influence on performance under the baseline conditions; in the case of k_3, q_2 is the only output showing some sensitivity. Figure 7.13 shows the time response of protein concentration in the product stream after step changes in feed, eluent and gel recycle flow rates.

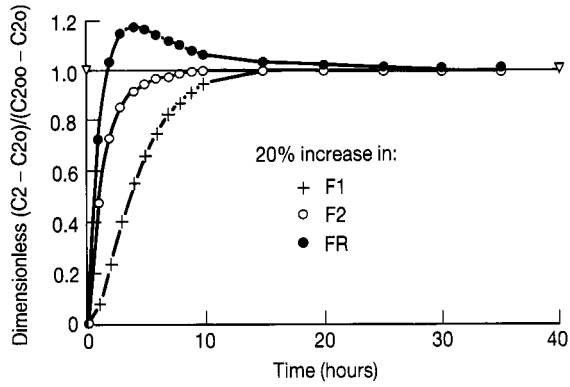

Fig. 7.13. Open-loop response of dimensionless production concentration to a 20% step change in input flow rates (Rodrigues *et al.*, 1992). F1 = feed flowrate; F2 = eluent flowrate; FR = recycle flowrate; C0, C1, C2 = protein concentration in streams shown.

In conclusion, a simple and effective process control scheme to regulate the continuous adsorption recycle extraction of proteins has been investigated. A dynamic mathematical model, based on unsteady mass balances, was derived and used in the study of the steady state and dynamic behaviour of the system, and to assist in the synthesis of appropriate control schemes. Sensitivity analysis showed that performance was strongly affected by the feed flow rate and feed protein concentration. The eluent flow rate was shown to be the most suitable manipulated variable. The protein concentration in the product stream was successfully controlled using a conventional PI (proportional integral) feedback controller, when measurement time delays are not significant (less than 50% of sampling interval). Model-based feedforward compensation further improved performance in those cases where the main disturbances can be measured on-line in the feed stream.

The control philosophy developed can be extended to other separation processes such as continuous two-phase aqueous separations featuring continuous liquid–liquid extraction (Asenjo *et al.*, 1991; Hustedt *et al.*, 1988).

7.4.3 Membrane chromatography

Chromatography is a well-used and well-understood method of purifying proteins. However, limitations in this process arise from mass transfer effects that occur in the beads. As a result of the relatively large diameter and porosity of the beads, diffusion times into the beads may be long, which results in broad peaks. In order to shorten the process time new efficient adsorbents are sought. One option was to reduce the bead size to minimise the effects of diffusive transport into the pores. This option gives rise to high pressure drops across the bed. A recent development is that of perfusion chromatography in which the adsorbents have many larger pores allowing greater flow velocities without compromising the resolution (Afeyan and Regnier, 1990). An alternative approach is to use membrane matrices as adsorbents. These have favourable characteristics such as high surface area, high solute throughput, mechanical strength, a highly porous structure and low pressure drops (Fig. 7.14). A potential advantage of membrane chromatography is its use in unclarified broth streams. There are also various process configurations which may be exploited. Membranes that are going to be used as adsorbents must have a high hydrophilicity, low non-specific protein adsorption, a uniform structure, a narrow pore size distribution, chemical and mechanical resistance and the ability to allow ligand coupling (Briefs and Kula, 1992). Ligand coupling may limit the modes of adsorption and types of interaction that may be suitable for membrane chromatography. Dye affinity ligands have been successfully coupled directly to the surface of nylon membranes; however, it was not possible to create an anion exchange membrane by this method and the use of a dextran spacer was required (Briefs and Kula, 1992).

Affinity membranes have been created by the coupling of triazine dyes to the membrane surface. These dyes have been successfully exploited as agents that mimic enzyme cofactors and other prosthetic groups, and thus may be used as affinity ligands. The technique of dye–ligand chromatography has been developed as a high-resolution purification technique.

The membranes that have been used are nylon-based (Ulitpore, Loprodyne, Immunodyne), and the dyes have been coupled to the membranes either directly or using

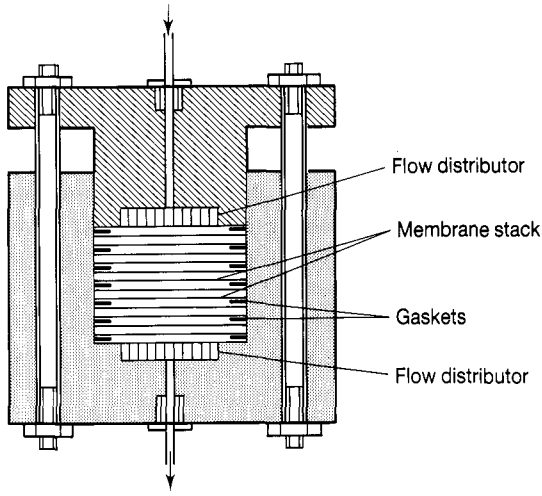

Fig. 7.14. Membrane chromatography system (Briefs and Kula, 1992).

1,6-diaminohexane or polyethylenimine spacers (Champluvier and Kula, 1990). These membranes are flat circular discs used in the laboratory for sample preparation.

Affinity membranes have been tested by batch adsorption and by membrane chromatography. In batch adsorption experiments pieces of dye-membrane are shaken with protein solutions in a test tube. In the membrane chromatography mode the dye-membrane discs were encased in a filter holder and protein solutions and buffers introduced with a syringe. Alternatively, up to 10 filter housings were stacked and connected to an FPLC apparatus (Pharmacia, Sweden) (Champluvier and Kula, 1991).

Experiments were carried out where the membrane–ligand system was compared with a bead–ligand system (Sepharose CL-4B) (Champluvier and Kula, 1990). Adsorption of malate dehydrogenase into Cibacron blue dye was performed. The loading of the protein was similar whatever the carrier. However, higher desorption of the protein was obtained with the beads. Further studies with the proteins adenylate kinase and glucose-6-phosphate dehydrogenase and beads of Sepharose CL-4B with yellow and blue dyes were carried out. The volumetric capacity of the membranes was similar to that of the gel beads. However, desorption was poor with respect to the Sepharose. The effect of the spacer was found to be very important. The dye concentration attached to the membrane was found to be five to ten times greater when polyethylenimine spacers were used as compared to directly binding the ligand to the membrane (Champluvier and Kula, 1991). Similarly, adsorption of lysozyme was found to be significantly higher onto dye-membranes rather than the non-specific binding to the undyed membrane.

Affinity membrane chromatography has been tested with the purification of glucose-6-phosphate dehydrogenase (G6PDH) from *Saccharomyces cerevisiae* as a case study (Champluvier and Kula, 1992). The ligand used was Cibacron blue F3GA bound to a Sartorius membrane with a thickness of 210 μm and pore size of 0.45 μm. The conclusions of this study were that the particles should be removed prior to adsorption to

assure fast operation and long-term performance. Adsorption of dilute enzyme solution was found to be very fast and results from the convective flow through the adsorbent and the fewer short diffusion pathways. Less than 25% of the bulk protein was adsorbed during the loading step, showing good selectivity. Desorption with KCl and PEG was slow and gave a trailing peak. This was improved dramatically by eluting with the cofactor NADP. Scale-up of this process to a membrane with a cross-sectional area 40 times greater was achieved. The eluted protein concentrations were similar as were the purification factors and yields. Scale-up may also be performed by stacking membranes, provided unacceptable pressure drops do not occur at the desired flow rates.

Other enzymes that have been purified by affinity membrane chromatography are pyruvate decarboxylase (PDC) from *Zymomonas mobilis* and formate dehydrogenase (FDH) from *Candida bodinii* (Briefs and Kula, 1992). Both enzymes are amenable to purification by dye-ligand chromatography; PDC was recovered using Procion yellow HE-4R and FDH by Procion red HE-3R. PDC was purified with an anionic membrane system and two gel matrices; DEAE-Sephacel and Mono-Q. It was found that the quality of resolution obtained by the membrane was better than the DEAE-Sephacel and nearly as good as the Mono-Q column. The cycle times for purification were shortest with the membrane, 1 minute as compared with 21 minutes for the Mono-Q column and 420 minutes for the DEAE-Sephacel system. As part of this study membrane chromatography was mathematically modelled. It was found that the kinetic effects leading to peak broadening can be neglected at flow rates less than 5 cm/min for affinity adsorption, and less than 20 cm/min for ion exchange. Secondly, the size of the pores has only a minor effect on resolution.

In conclusion it is evident that the high flow rate and high efficiency of capture characteristics of membrane chromatography are well suited for processing large volumes of dilute protein solutions. Choice of membrane will be very important. It was found that the available area for binding was not dramatically reduced when membranes with larger pore sizes were used. Pores with diameters of 1.2–3.0 μm are suitable for low pressure, high capacity and good resolution.

7.4.4 Chromatographic and adsorption materials

Perfusion chromatography represents an interesting approach to overcoming speed and throughput limitations in chromatography caused by diffusive mass transport. The packing used for perfusion chromatography contains two classes of pores: *throughpores* (see Fig. 7.15) to allow convective flow through the particles and smaller, *diffusive pores* lining the throughpores to provide high adsorption surface area. Conventional materials contain only diffusive pores and thus solute transport to the binding sites on the medium is limited by the slow diffusion process.

In perfusive media, when the mobile phase reaches a sufficiently high velocity, convection in the throughpores takes place. In this regime, the combined rapid convective transport and ultra short path length in the diffusive pores make resolution and dynamic loading capacity virtually independent of flow rate with linear velocities, ca. ten times or even higher than with conventional materials.

Inverted matrix chromatography uses a resin matrix where the resin is the continuous phase in the matrix and the void spaces are distributed. This is in contrast to the normal

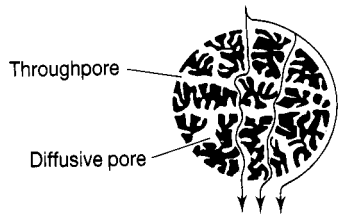

Fig. 7.15. Perfusion chromatography.

resins which consist of discrete particles. Very low pressure drops are possible and their use in direct broth extraction is presently being investigated (Howell *et al.*, 1993).

Affinity chromatography has been used for a number of years both at the laboratory as well as at the large scale. Most early industrial processes for therapeutic proteins included one or more affinity steps. This corresponds to a very high-resolution purification even in the absence of physicochemical knowledge about the main contaminants (see Table 7.1). These, however, relied in many cases on very large ligand molecules (e.g. antibodies) and were usually exorbitantly expensive at either the large scale or even the laboratory scale. At present there is a tendency to use less expensive, more 'generic' ligands. Important advances in this area have been obtained in the last few years which include the synthesis and use of specific *dyes* and also of *metal ions*. This technique is also known as immobilised metal affinity chromatography (IMAC).

7.5 OTHER DEVELOPMENTS

7.5.1 Electrically enhanced separations

Electrophoresis is capable of resolving biological molecules on the basis of differences in their molecular weights, isoelectric points and mobilities in an electric field with a very high resolution. At the laboratory scale this includes some of the most powerful techniques available for the purification of biologically active molecules. Isoelectric focusing (IEF) and recycle isoelectric focusing (RIEF) are used in the laboratory as efficient preparative techniques to fractionate complex protein mixtures. The main advantage is the very high resolution; however, removal of ampholites and the low protein solubility at its isoelectric point are disadvantages for large-scale use. Other techniques that have been at least partially scaled up are recycle continuous flow electrophoresis (RCFE) and counteracting chromatographic electrophoresis (CACE) (Asenjo, 1990).

As electrophoresis is seldom employed at the commercial scale primarily due to the convective mixing problems caused by ohmic heating it has recently been shown that an aqueous two-phase system can be used as a medium for electrophoretic transport and that the liquid–liquid interphase provides stability against convection and facilitates product recovery. The method combines the scalability of aqueous two-phase systems with the selectivity of electrophoresis.

Electrically enhanced membrane processes are presently being investigated as potentially useful techniques both for resolution of protein mixtures and for the separation of disrupted microbial cells (Brors *et al.*, 1993; White *et al.*, 1993).

7.5.2 Genetic approaches to protein purification

Recombinant DNA techniques have also allowed the development of highly specific separations. This has been carried out by adding a specific tail to the target protein by genetic engineering techniques (e.g. the zz domain of protein A) which binds a specific ligand (e.g. IgG or a more inexpensive ligand such as metal ion). After purification of the protein the tail is removed usually by enzymatic cleavage.

7.5.3 Purification of intracellular proteins

A large number of proteins synthesised in *Escherichia coli* and yeast are intracellular. These include intracellular protein particles such as recombinant vaccines. Hence the first step in their separation consists of their extraction from the contaminant cell material. Techniques for *selectively* carrying out the *solubilisation* or *extraction* of the protein are presently being developed (Fig. 7.16). These include the use of solvents (e.g. toluene), chelating agents (e.g. EDTA), detergents (e.g. Triton X-100) and chaotropic agents (guanidine and urea). Also, the use of a pure lytic glucanase to selectively release recombinant 60 nm protein particles (virus-like particles or VLPs) from yeast has recently been reported (Asenjo *et al.*, 1993). The VLPs have been used in trials to manufacture an AIDS vaccine already for 3 years. When using the crude lytic complex which in addition to glucanase contained some lytic protease, the protease components of the complex were found to degrade the VLPs. The purified glucanase enzymes from these complexes produced cell lysis without degradation of the VLPs. The *Oerskovia* lytic glucanase enzyme released the recombinant protein particles selectively as it only produced ca. 17% cell lysis compared to the use of the crude lytic enzyme preparation (with lytic protease). This selectivity, which results in the release of the recombinant particles with only a fraction of contaminating proteins, represents an improvement over presently used mechanical or enzymatic cell disruption processes. This pure lytic glucanase is presently being cloned in *E. coli* and *Bacillus* (Ferrer *et al.*, 1993) strains used for commercial production of extracellular enzymes. The large-scale availability of

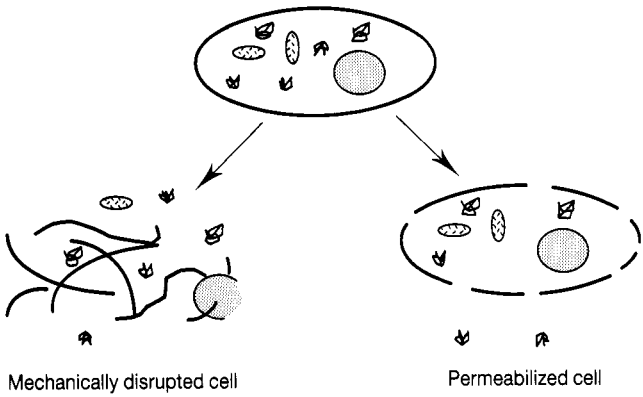

Fig. 7.16. Conceptual comparison of mechanical disruption and permeabilisation for obtaining intracellular product release.

an inexpensive lytic $\beta(1-3)$ glucanase will find use in improved processes for selective recovery of intracellular proteins from yeast.

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Chapter 8

Fractionation of fat

KANES K. RAJAH, Welsh Institute of Rural Studies, University of Wales, Aberystwyth, Penglais Campus, Aberystwyth, Dyfed SY23 3DD

8.1 INTRODUCTION

Edible fats are derived from animal, marine or plant sources. They are often not available in edible form and in forms whereby they could be used readily in food preparations. The recovery of fats from their source is usually through rendering (animal, marine), crushing (seed oils), cold expression (speciality seed oils) and churning (as in butter from dairy cream). In most instances animal, marine and vegetable fats are processed further through degumming, bleaching, neutralisation and alkali refining or physical refining, and finally deodorisation before the fat is declared satisfactory for edible use – i.e. as a bland, odourless, pale and clear fat with good shelf-life stability. However, before this final stage is reached the fat would have undergone some form of separation during one or several stages of process, starting initially with filtration to remove impurities.

A number of these edible fats are treated further, through hydrogenation, interesterification and fractionation to improve their oxidative stability, nutritional and functional value and processing properties (Moran and Rajah, 1994; Rastoin, 1985). For instance, stability is improved by light hydrogenation, fractionation can be used to remove a proportion of high melting components (more saturated triacylglycerols) for use as pastry fats (e.g. milk fat stearin), and the sandy texture arising from using non-modified lard in margarine can be improved by interesterification (Hannewijk, 1972).

Some hydrogenated or interesterified fats are themselves in turn fractionated to recover the appropriate fraction(s) for specific food applications. Hydrogenation and interesterification reactions are mostly followed by separation processes, to remove, for instance, nickel catalyst from the hardened fat.

Fractionation, however, is primarily a separation process, where fat is first nucleated and crystallised and then separated from the liquid phase using one of several techniques. This chapter will deal mainly with the fractionation of edible fat. The products from fractionation and their application in food are outside the scope of this book but this subject has benefited from a comprehensive review in a recent publication (Rajah, 1994).

The fractionation of edible oils and fats was practised as early as the mid-nineteenth century when oleomargarine was made from fractionated bovine tallow.

This early manufacture of margarine was based on the invention of the French chemist, Hippolyte Mege Mouries (in 1869). He first obtained fresh tallow by careful rendering. The purified fat was then submitted to a slow crystallisation process at about 25–30°C (Andersen and Williams, 1965). The grainy coarse product which resulted was hydraulically pressed and yielded about 60% of a soft semi-fluid yellow fraction, oleomargarine, and about 40% of a hard white fat, oleo-stearine. The softer fraction had approximately the same melting point as milk fat and could be easily plasticised. Mouries also believed that the soft part consisted of margarine and olein, the acylglycerols of margaric and oleic acids respectively, and the crystalline material mainly of the acylglycerols of stearic acid. Hence the name oleomargarine for his new butter-like product.

The advantages of fractionation were first appreciated in Europe by the importers of coconut oil from Sri Lanka (Rossell, 1985). Warm fluid oil which was filled into long wooden barrels called 'Ceylon Pipes' cooled slowly as it sailed towards the cooler European climate and, perhaps aided also by the gentle agitation of the ship's movement, crystallised and separated into fractions. This partly crystallised fat was evaluated by the recipient fat companies who found that the stearin fraction could be used to advantage in the couveture and coatings industry.

When commercial scale fractionation first commenced, the process of cooling took place in large wooden vats, agitation being a manual operation using paddles. The crystalline suspension was separated by filtration through cloth. The stearin was then collected, wrapped in cloth, and squeezed in tower presses to increase the olein yield. However, the fractionation of fats soon declined and in the years following World War I it virtually ceased. Meanwhile, although the consumption of margarine rose and with it the demand for the hard base stock, this need was satisfied by the then fast developing hydrogenation industry using the process invented by Senderens and Sabatier in 1902. Hardened, or hydrogenated fats, mixed with liquid vegetable oils and non-fractionated bovine tallow enabled the formulation of the base stock for margarine, and remains so as we know it even today. During that period the small quantities, i.e. 2–5%, of wax or stearin recovered from the winterisation of salad oils such as sunflower oil and cottonseed oil were also processed into the margarine oil blend.

The revival of fat fractionation finally came during the mid-1960s, following the remarkable upsurge in palm oil production, particularly in Malaysia. It provided the impetus to many to review the principles, processes and techniques on the subject. It also aroused the interest of the international dairy industry, and they too studied the technology to seek new opportunities for milk fat. The principle of the fractionation process can be described schematically as shown in Fig. 8.1.

Three major commercial processes are available for the fractionation of fats. These combine the crystallisation (Saxer and Fischer, 1983) and separation processes:

(1) *Dry fractionation.* The crystallisation stage can be either rapid or slow and crystals are separated through direct filtration i.e. without the use of additives.
(2) *Detergent fractionation.* Crystallisation is generally rapid and an aqueous solution

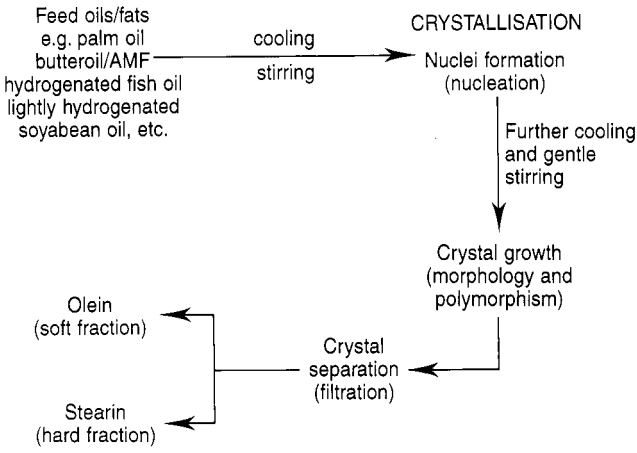


Fig. 8.1. The fractionation process.

containing detergent is used to facilitate the separation of the crystals from olein (Lipofrac) by centrifugation.

(3) *Solvent fractionation.* The crystallisation is carried out in solvents followed by filtration. This process is not used widely due to its high operating costs except for the production of high value products such as cocoa butter replacer. It will therefore only receive a brief treatment in this chapter.

8.1.1 Crystallisation: nuclei formation and crystal growth

The controlled cooling of molten fat slows the thermal motion of the molecules, drawing them closer together through intermolecular forces, whilst simultaneously enabling parallel ordering of the fatty acid chains to take place. As a consequence nuclei form and crystallisation commences. Here, if the probability of a molecule being absorbed exceeds that of a molecule being liberated, then these molecular aggregates will grow into real crystals. Cooling aids the absorption of molecules by lowering the potential energy. The nucleation rate increases until a maximum is reached (Tammann, 1903) but further cooling contributes to a reduction in nucleation rate because the viscosity of the melt is increased, which as a consequence reduces the rate of diffusion.

Mortensen (1983) reported that when formed, milk fat crystal nuclei grow through the deposition of successive single layers of molecules on an already ordered crystal surface. The probability of the incorporation of these molecules into the crystal lattice as well as the material density and the temperature, which influences the rate of diffusion, all play a primary role in the rate of growth of the crystals. It is also evident from studies using milk fat that for a given reactor vessel with a fixed rate of agitation (stirring), the cooling rate, i.e. rate of temperature drop, determines final crystal composition (Rajah, 1988):

(1) Rapid cooling rates resulted in high yields of crystals with low N_{20} values (solid fat index values at 20°C) due to entrapped olein.

(2) Moderate cooling rates promoted the development of crystals with primarily high melting triacylglycerols and high N_{20} values, although the yields were somewhat reduced.

(3) Slow cooling rates resulted in high yields of crystals, but the solid fraction had reduced N_{20} values. This is interpreted as being due to the development of nuclei which allow growth of crystals comprising high and medium melting triacylglycerols.

Deffense (1985) observed that in factory operations filtration units cannot compensate for poor quality crystals, the latter being formed as a result of rapid cooling; the crystals group together and form clumps within which part of the liquid phase is occluded. Hence there is a decrease in olein yield of up to 10%. Deroanne (1975) reported in his dissertation that a low yield could also result from intersolubility and the formation of mixed crystals.

8.1.2 Polymorphism

Crystals can exist in three main forms, α-, β'- and β- (Chapman *et al.*, 1971) in order of increasing stabilities and melting points. A metastable α-form results upon rapid cooling and is produced reversibly from the liquid phase. Hence rapid supercooling can result in a mass of very small crystals. In general the oil crystallises into the unstable α-form and then rapidly transforms into the more stable β'-form, and much more slowly into the β-form. However, Hoerr (1960) stressed that less pure samples and triacylglycerols with a more complex composition may exhibit intermediate forms which are difficult to identify. Deffense and Tirtiaux (1982) reported that when crystals are in the β'-form they are firm and of uniform spherical size and hence are easy to separate from the olein phase. For palm oil these β'-crystals should be near 0.1 mm in size (Deroanne, 1976). β'-crystals are formed readily when the oil is free from crystal inhibitors such as gums, carbohydrates, soap, mineral acids and monoglycerides.

8.1.3 Quality of edible oils

When edible oils are freshly recovered from their source they are generally referred to as crude oils. There are some notable exceptions, e.g. dairy cream, olive oil, which is preferred in its untreated form so that its delicate flavour is retained, and even palm oil is consumed in the crude form in some parts of Africa. Most crude oils, however, contain impurities which need to be removed to make them palatable. The major processes used in removing these impurities are degumming (if the crude oil is of plant origin), chemical and physical refining (to reduce free fatty acids), bleaching (to remove much of the colour) and deodorisation (to remove flavour taints and other volatile material contributing to odour). Removal of impurities from the feed facilitates the fractionation process, particularly filtration throughput. For comparison, during fractionation, a Tirtiaux Florentine filter type FLO 1000 will have a throughput of 2 tonnes/h on crude palm oil, 6 tonnes/h on refined palm oil, 8 tonnes/h on olein and 11 tonnes/h on beef tallow (Tirtiaux, 1980). The impurities also affect some laboratory analyses. For instance it has been reported (Haraldsson, 1978) that when crude palm kernel oil was used in the Lipofrac process to produce hard butters, the ensuing dilatation curves showed the refined stearin to have higher dilatation values, and a higher melting point, when compared to the crude stearin, Fig. 8.2. He attributed this to the removal of the free fatty acids

during refining, as the free fatty acids in a way function as a solvent on some high-melting acylglycerols. Similar behaviour was noted for the olein fraction.

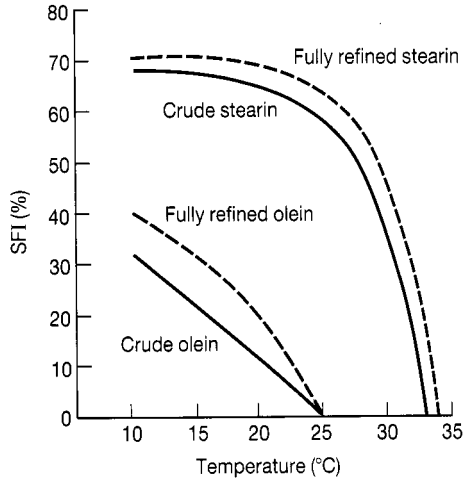


Fig. 8.2. Influence of refining on dilatation of PKO fractions (Haraldsson, 1978).

8.2 DRY FRACTIONATION

Dewaxing and winterisation (Thomas III, 1985) are two limited forms of dry fractionation used for the removal of waxes and high melting triacylglycerols respectively from liquid vegetable oils. For instance, sunflower oil and corn oil contain a small proportion of waxes which give them a cloudy appearance at refrigerated temperatures. While others, such as cottonseed oil, contain triacylglycerols which are rich in saturated fatty acids. Dewaxing and winterisation, respectively, make these oils suitable for use as salad oils and for use in emulsions like mayonnaise. In the case of the latter, without this treatment, the high melting triacylglycerols would crystallise and separate during storage, causing the emulsion to break.

Cottonseed oil, however, has substantially more palmitic fatty acid, in the range 17–29%, compared with other liquid vegetable oils. This saturated fatty acid which, present as palmitic-linoleic-palmitic (PLP) triacylglycerol in cottonseed oil, crystallises out at normal ambient temperatures. This was recognised very early on in the United States, where cottonseed oil is widely available, and was as a consequence the first oil to be winterised.

If the stearin is not removed, the cottonseed oil partially solidifies when stored at temperatures below 10–15°C.

Nowadays, winterisation of cottonseed oil is carried out at refrigeration temperatures. The amount of stearin formed can be large. Although yields can be well in excess of 20%, filtration is still quite rapid. In view of this, during dry fractionation–winterisation processes, such as that offered by CMB Bernadini of Italy, the crystallisation is carried out in several large horizontal crystallisers, Fig. 8.3, so that, when ready, enough feed is

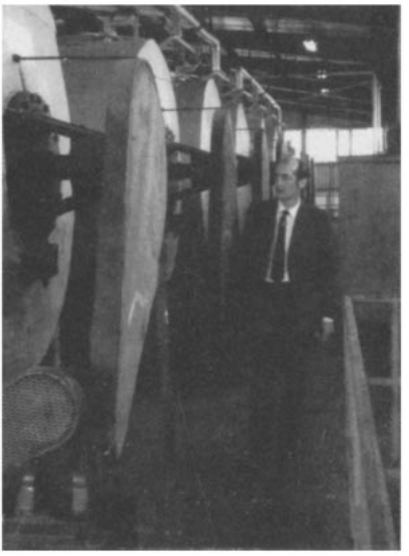


Fig. 8.3. Winterisation of cottonseed oil using horizontal crystallisers (courtesy of CMB Bernadini, Italy).

available for continuous filtration. If suitably prepared, the melting points of these stearins can be in the range 20–25°C (Rossell, 1994).

Cottonseed oil stearins can be an important source of zero trans fats which can substitute for hydrogenated fats, the latter being the subject of some concern in relation to trans fatty acids in the diet (Applewhite, 1994). They may find application in a variety of food formulations including margarines, soups and sauces. With annual world consumption of cottonseed oil currently at about 3.5 M tonnes, this potentially large source of zero trans fat is not being fully exploited. Ironically, much of the stearin from cottonseed oil goes into blends with soyabean oil which is then hydrogenated into hard stock for margarine and shortening manufacture. Since it is the hydrogenation reaction which is the main cause of trans fatty acids in processed fats, fractionation could well gain further importance as a means of generating zero trans hardstock, such as stearins from palm oil fractionation with melting points typically in the range 40–50°C (Rossell, 1994).

Fats which contain a large proportion of higher melting triacylglycerols, e.g. milk fat, palm oil and tallow, are treated to full fractionation where both fractions, i.e. stearins and oleins, are recovered in large amounts, typically 20–30% stearin and 70–80% olein. Although manufacturers of fractionation equipment offer complete systems incorporating both the crystallisation tanks as well as the filtration units, the processes are generally referred to by the filtration system selected.

Three major filtration routes are available:

(a) flat-bed vacuum band filter;
(b) rotary drum vacuum filter;
(c) membrane, positive pressure filter.

8.2.1 Flat-bed vacuum band filter

Florentine continuous filter

The Tirtiaux process (Tirtiaux, 1980; Ricci-Rossi and Deffense, 1984) for fractionation of fats by gradual and selective cooling followed by filtration on their patented, continuous belt, 'Florentine' vacuum filter is probably the most widely used flat-bed system. Crystallisation is normally carried out in two stages (Kreulen, 1976). The plant layout includes a precrystallisation stage where the feed is first cooled slightly to reach nucleation. It is then pumped into crystallisation tanks. The vertical tanks are jacketed and fitted with an agitator or a coil or both, depending on their size, varying between 12 and 50 tonnes. The agitator provides convection movements without scraping the wall during the whole cooling stage for efficient crystallisation. The oil is cooled under controlled conditions, whereby it is the temperature of the oil that actually controls the rate of cooling. This slow crystallisation enables control of the latent heat of crystallisation and avoids supercooling. When crystallisation is complete and the filtration temperature is reached, the slurry is filtered on the Florentine continuous-belt filter (Fig. 8.4).

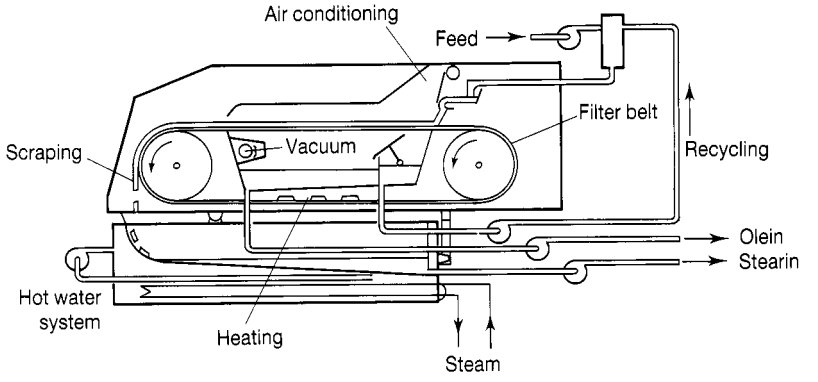


Fig. 8.4. Florentine filter (courtesy of Tirtiaux, Belgium).

The Florentine is a horizontal type, flat-bed filter. The filtration takes place on a continuous perforated stainless steel belt operating at a vacuum of 50–200 mbar. The filter is fitted with a recycling device which enables the filtrate from the first filter section to be recycled. This facilitates filtration on a preformed stearin cake and increases the quality of the filtrate. The filter is self-cleaning, and the filtration area is enclosed and air-conditioned. The latter helps to maintain the feed slurry at the temperature of fractionation until separation of the fractions is completed. Filtration is possible at temperatures as high as 45°C (tallow) or as low as 2°C (lightly hardened soyabean oil).

The Tirtiaux process was first developed in Europe for the fractionation of beef tallow. This was later extended to palm oil when they installed the first commercial plant in

Bogota in 1969. Subsequently other plants were installed world-wide to fractionate a variety of oils including milk fat, lard, hardened (hydrogenated) soyabean and fish oils.

Yields of oleins are typically in the range 67–72%, and Deffense (1991) has suggested a further 8% increase is achievable if a membrane filter is used. The Florentine filter can be used to separate high levels of solids, up to the 60–70% range if required.

Vacuband batch filter

The Miller Vacuband filter (Miller, 1980; Kehse, 1979) which is a stationary bed vacuum filter (see Fig. 8.5; Rajah, 1988), was used in semi-commercial scale production of milk fat fractions. This system, offered by CJC (Oakmere, Cheshire, UK), shows important advantages over the 'open' vacuum systems.

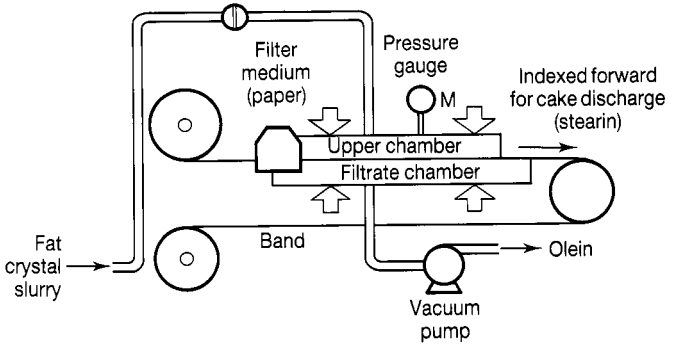


Fig. 8.5. Vacuband filter arrangement (Miller Filter Company Ltd, Overath, Germany and Chris James Consultants, Oakmere, Cheshire, UK).

Crystallisation of milk fat was carried out in a jacketed stainless steel vessel of virtually identical design to that of a batch stirred tank reactor (BSTR). Nominal working capacity of the vessel was approximately 400 kg charge of anhydrous milk fat (AMF) feedstock. To achieve good heat transfer characteristics, the vessel was fitted with a variable speed, full sweep, anchor-type agitator arranged to prohibit mass rotation. Agitator speeds were possible within the range 4–30 r.p.m. although the optimum range was found to be 7–10 r.p.m., i.e. 0.36–0.52 m s^{-1}. The vessel was additionally rated at 3.3 bar, for positive pressure nitrogen blanketing of product. During crystallisation, the head space was purged to establish a nitrogen blanket. The temperature difference between the oil and water jacket was maintained at a maximum of 5°C. Separation of milk fat crystals was carried out on the novel, stationary-bed, vacuum band filter, the Vacuband, surface area 1 m^2, Fig. 8.5. The novelty lies in being able to filter and separate the liquid from the solid phase, under vacuum, within an enclosed upper chamber. This solid–liquid separation system is being used in a variety of liquid processing industries and in the edible oil industry during bleaching earth filtration, winterisation, and hydrogenation catalyst filtration. The unit comprised an indexing, horizontal rolled stored filter medium (paper), arranged over a static lower vacuum chamber and with a second upper movable (vertically) vacuum/feed chamber in opposition. The standard design utilised the upper

chamber to recreate a self-feeding system using upper chamber vacuum level, and on completion of each filtration cycle the vacuum in each chamber was released, and the upper chamber opened by lifting up, allowing the band to be indexed forward to its discharge. A stainless steel wire, fixed along the width of the band, ensured that the cake was dislodged from the filter paper and dropped into the heated trough in front of the filter. When the cake liquefied it was transferred via a butterfly valve at the base for packaging or texturisation for food use. The filtrate (olein) drawn under vacuum during filtration, was transferred via an intermediate vacuum tank, filtrate receiver, into the filtrate storage tank before being drummed. The most suitable filtration medium was found to be Paper/Binzer Type 67/N, 80 g, roll, 0.108 m in width and approximately 200 m in length, of bleached crepe quality.

The yields for milk fat were typically 76–80%, Table 8.1, compared to 67–72% (Deffense, 1985) for the Florentine filter. This is attributed to the improved efficiency achieved by using the integral, vacuum sealed, upper chamber. The fastest crystallisation rate was established as 6°C h^{-1}, cooling down to 28°C for satisfactory filtration.

Laboratory analyses carried out on milk fat fractions from vacuband filtration are given in Table 8.1 (Rajah, 1988). Comparative results on products using rotary drum and membrane press filter are given in Table 8.2 (Kokken, 1992). (*Note*: The 'Drop point' is a measure of the melting point of the oil or fat relating to the temperature at which an oil drop falls freely when a solidified sample is warmed in a cup with a small hole.)

The multi-step fractionation of milk fat was also carried out using the vacuband filter. In this type of process the oleins from successive fractionations are used as feed for further fractionation. Typically, the quantity and size of crystals is maximised when oleins are cooled to temperatures of between 2 and 5°C below their melting point. Using this route, two-, three- and four-step fractionations have been completed satisfactorily, Table 8.3 (Rajah, 1988). In low temperature fractionations it is important to ensure that environmental temperatures are carefully controlled and that all contact surfaces for the crystal slurry are held at the temperature of separation. Low melting point milk fat oleins can be used in food applications where only liquid oils are normally used, e.g. mayonnaise (Rajah *et al.*, 1984).

8.2.2 Rotary drum filter

De Smet supply complete fractionation plants incorporating the 'Stockdale' type rotary drum filter.

The crystallisation step is quite rapid, an average maximum of a 6 h cooling cycle is common. However, in order to ensure efficient and effective crystallisation the design of the crystallisation tank has to include a large cooling surface with good agitation facility. Typically, industrial crystallisers capable of holding up to 25 m^3 product are presently available with these features. To achieve homogeneous supersaturation of the oil during cooling and even temperature throughout the mass of the oil, the distance between each crystal and the cooling surface must be minimised to enable the efficient dissipation of the heat of crystallisation. For this reason the use of a two-speed motor, with variable-speed gearbox, or if possible a continuous variable-speed motor, is proposed to drive the agitator. At the start of the process when the oil is in the molten state, at higher temperature (65–70°C), maximum agitation increases heat transfer and consequently

Table 8.1. Milk fat fractionation using Vacuband filtration. (Filtration temperature: 28°C) (Rajah, 1988)

	Crystallisation time (h)	Yield (%)	Melting point (°C)	Iodine value (IV)	Solid fat content (SFC)			
					10°C (%)	20°C (%)	30°C (%)	40°C (%)
(1) Fast crystallisation								
Feed (anhydrous milk fat)	8.4		34.7	30.1	53.0	20.1	5.6	—
Olein		76	23.5	33.0	45.2	10.7	0.1	—
Stearin		24	42.3	25.6	69.6	43.0	23.7	4.3
(2) Slow crystallisation								
Feed (anhydrous milk fat)	17.8		32.5	39.9	49.9	16.4	4.4	—
Olein		80	22.0	42.6	39.9			—
Stearin		20	44.4	26.4	80.1	61.1	40.6	13.1

Table 8.2. Milk fat fractionation using (1) rotary drum filter and (2) membrane press filter, at a filtration temperature of 25°C (Kokken, 1992)

	Yield (%)	Drop point (°C)	Melting point (closed capillary method (°C)	IV	SFC 10°C (%)	SFC 20°C (%)	SFC 30°C (%)	SFC 40°C (%)
(A) Feedstock	—	33.3	35	32.72	32.72	16.12	4.4	0.22
(B) 6 hours crystallisation time								
Rotary drum filter								
S25	45.6	38.3	39.8	29.06	50.74	30.93	13.72	0.11
L25	54.4	21.5	23.5	34.25	28.81	2.58	0.13	0.05
Press filter								
S25	32.1	41.2	42.0	27.23	61.17	41.88	22.51	4.3
L25	67.9	21.2	22.8	34.91	29.06	1.85	0.15	0.11

shortens the cooling time. Once crystallisation commences, the agitation rate has to be decreased to avoid secondary nucleation caused by the fragments of crystals fractured by excessive agitation. If the latter happens control over the crystallisation is lost, since this leads to the formation of a very large number of small crystals which consequently lead to gel formation. In the crystallisation of palm oil De Smet have also observed (Kokken, 1988) that too low an agitation rate leads to local overheating from the exothermic crystallisation reaction. This leads to dissolution of nuclei which are not sufficiently close to the cooling surface. It also leads to a high degree of supersaturation in the vicinity of the cooling surface, thereby leading to the inclusion of olein in the crystal structure which results in weak crystals. The specially designed and patented oil cooler–crystallisers enable crystallisation to be completed in less than half the time taken with the typical Tirtiaux process (Figs. 8.6, 8.7).

The rotary drum vacuum filter is essentially a multi-compartment filter, consisting of a drum rotating about a horizontal axis and so arranged that the drum is partially submerged in the trough holding the oil slurry (Fig. 8.8; Filtration Services Ltd, Macclesfield, England). The periphery of the drum is divided into compartments, each of which is provided with a number of drain lines; these pass through the inside of the drum and terminate as a ring of ports covered by a rotary valve. It is by way of this valve that vacuum is applied. The surface of the drum is covered with a filter fabric and the drum is arranged to rotate at low speed, usually in the range 0.1–0.25 r.p.m. but up to approximately 3 r.p.m. for very free-filtering materials.

Table 8.3. Multi-step fractionation of milk fat using the Vacuband filter. Analytical data for (1) two-, (2) three- and (3) four-step fractionations (Rajah, 1988).

	(1) Two-step fractionation			(2) Three-step			(3) Four-step		
	Olein (feed)	Olein	Stearin	Olein (feed)	Olein	Stearin	Olein (feed)	Olein	Stearin
Carotene (ppm)	8.5	9.5	7.3	8.2	9.6	7.2	9.9	10.8	8.4
Iodine value	41.5	46.5	40.5	44.5	48.7	41.7	44.8	48.7	41.2
Melting point (°C)	25.3	11.9	25.1	13.0	8.4	13.7	9.6	9.6	13.2
Solid fat content (%)									
N_0	50.8	37.0	59.1	37.2	21.2	45.6	25.0	14.7	42.1
N_5	45.9	29.6	54.9	29.6	12.7	39.2	17.5	7.3	34.4
N_{10}	37.5	17.3	47.5	17.6		28.4			17.0
N_{15}	19.2	0.6	31.0	0.4		8.4			0.7
N_{20}	5.6		12.9			0.2			
N_{25}	1.5		4.4						
N_{30}			0.4						
Fatty acids (%)									
14:0	9.8	8.6	9.8	9.7	10.8	11.1	9.2	9.0	10.0
16:0	21.2	17.1	22.3	21.8	16.9	21.4	19.0	17.3	21.7
18:0	10.4	7.4	11.0	8.3	6.6	8.5	7.7	6.7	8.9
18:1 trans	4.3	4.1	4.0	3.6	3.8	3.3	4.4	4.4	4.4
18:1 cis	22.8	23.1	20.6	27.1	28.6	24.4	31.4	32.1	27.9

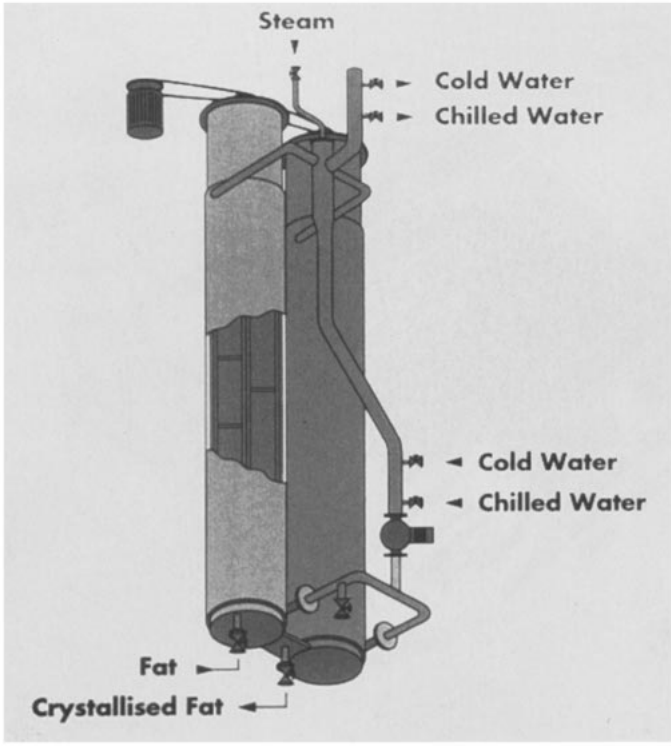


Fig. 8.6. Oil cooler crystalliser (outside view, courtesy of De Smet Rosedowns, Belgium).

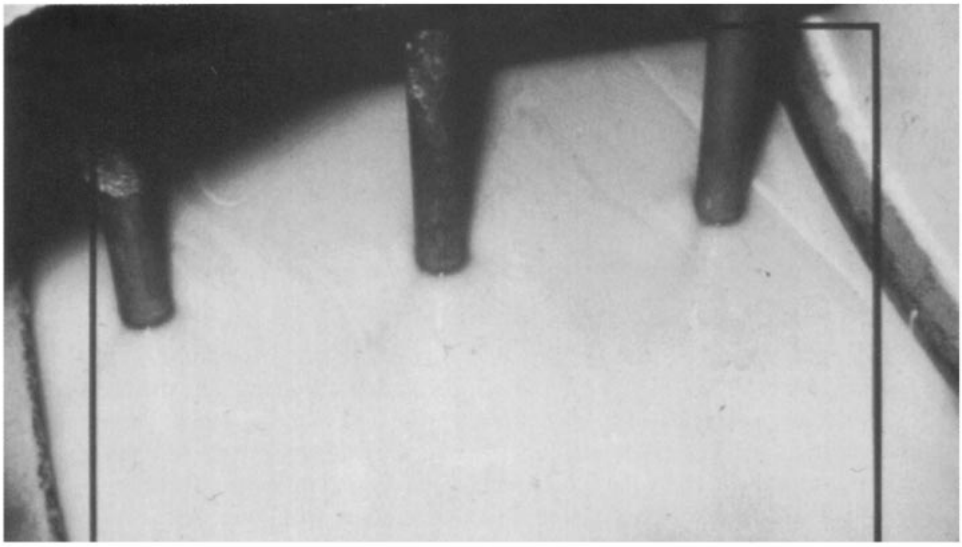


Fig. 8.7. Oil cooler crystalliser (inside view, courtesy of De Smet Rosedowns, Belgium).

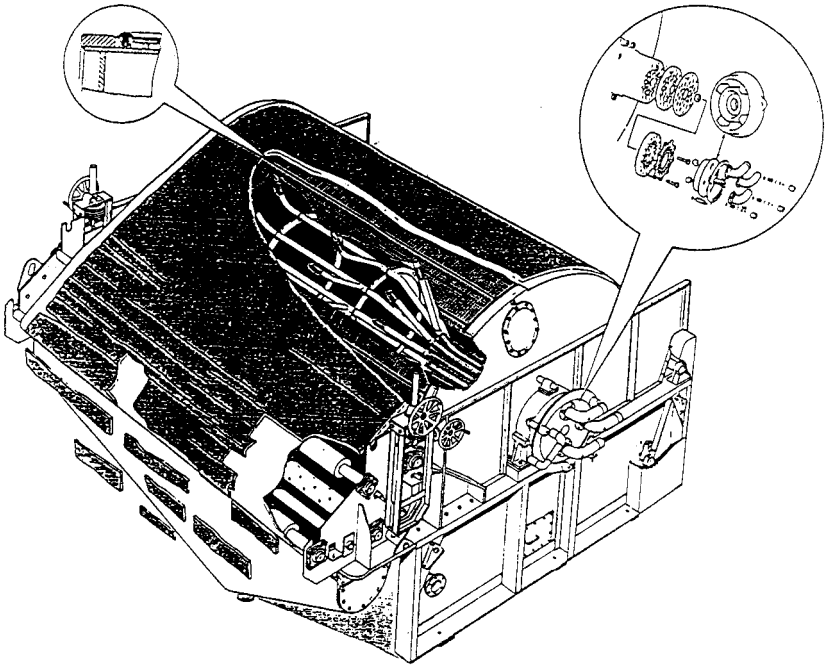


Fig. 8.8. Stockdale belt discharge vacuum filter (courtesy of Filtration Services Ltd, Macclesfield, UK).

As the drum rotates, each compartment undergoes the same cycle of operations, the duration of each of these being determined by the drum speed, the submergence of the drum and the arrangement of the valve. The normal cycle of operations consists of filtration, drying and discharge.

The rotary drum vacuum filter shown in Fig. 8.9 is the 'belt discharge' unit. The filter fabric leaves the drum carrying solids and is washed after the cake is discharged and is then returned to the drum. In the De Smet system the oil is continuously filtered through a nylon cloth, and stearin is discharged by compressed air blowing through the nylon cloth, combined with scraper action.

The capacity and suction pressure of the vacuum pump is selected by consideration of the size of the filter and the permeability of the cake. For many fractionation operations this is about 0.6 m^3 min^{-1} m^{-2} at –0.6 bar G (or 0.4 bar absolute). The diameter of the vessel or receiver is calculated to give a low vapour velocity, i.e. 1 m s^{-1}, to allow the liquid to dis-entrain. The vessel height is determined by the volume of oil to be retained, which in turn is influenced by the filtrate pump capacity and its control (Bosley, 1994).

More than 60 of the 'Stockdale' filters are in operation world-wide (current suppliers, Filtration Services Ltd). They are being used for the dry fractionation of palm oil, and other vegetable oils and fish and animal fats.

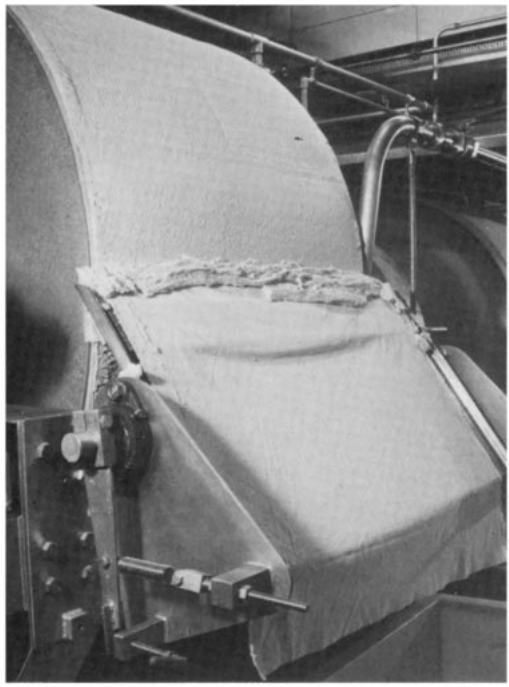


Fig. 8.9. Stockdale rotary drum vacuum filter (courtesy of Filtration Services Ltd, Macclesfield, UK).

A rotary drum vacuum filter system by Nivóba is understood to be in operation in France for the production of milk fat fractions. This design makes use of smaller crystallisation tanks but with dimple plate internal walls for increased surface area to volume ratio. A similar filtration system was used by Schaap and van Beresteyn (1970) in their pilot scale studies on milk fat.

In the CMB (Costruzioni Meccaniche Bernadini, Pomezia-Rome, Italy) dry fractionation process the feed oil is first crystallised in batch crystallisers, each fitted with three thermo-regulated water tanks held at different temperatures. Cooling commences when water from each tank is circulated in sequence for a pre-determined time period to facilitate fast cooling of the oil. Palm oil, for instance, is thermoregulated from 35°C down to 16°C before crystals are ready for separation. The filter system comprises a slowly rotating horizontal shaft from which separate vacuum filter elements fan out in a radial arrangement. The unit is dipped in a chamber containing the free slurry, which is held at constant level. As the elements become submerged in the oil slurry, olein is sucked in and a coating of stearin forms on the surface which is expelled by compressed air.

8.2.3 Membrane filters

Low pressure

The crystallisation stage can be either rapid or slow and along similar lines to that described for flat-bed or rotary drum vacuum filtrations. The membrane filter is manufactured by a number of companies, including Hoesch, Tirtiaux and De Smet, and the main principle of operation remains the same. The Hoesch membrane filter was the first to be tested commercially during 1982–84, for palm oil fractionation in Malaysia. It is a technological advance on the time-tried chamber filter press, an external distinguishing feature being air-supply hoses to every other filter plate. The principle of the operation is shown in Fig. 8.10. The plates are equipped on either side with a flexible diaphragm or membrane. When compressed air is introduced between the plate and diaphragms it exerts static pressure on the filter cake enveloped within the filter cloth and squeezes out the olein against a flat plate on the opposite side of the cloth. Initially, when the cloth is clean, filtration takes place at 0.1–1.0 bar. As fouling of the cloth through repeated filtration becomes more significant a much higher pressure is required, in excess of 2.0 bar, up to about 5 bar. A typical membrane would be made of oil-resistant and food-compatible natural rubber combined with neoprene rubber on account of its flexibility. The membrane is not rigidly connected to the filter plate. Instead it is attached inside a dovetailed groove to the plate and can therefore be easily replaced. This also means that any accidental addition of compressed air, when the press is open, would not cause it to tear but merely loosen it from its fixture. Tirtiaux addressed this problem by fitting a control mechanism which ensures that squeezing only takes place when all the chambers are filled with slurry. However, Hoesch claim that their membrane is capable of such

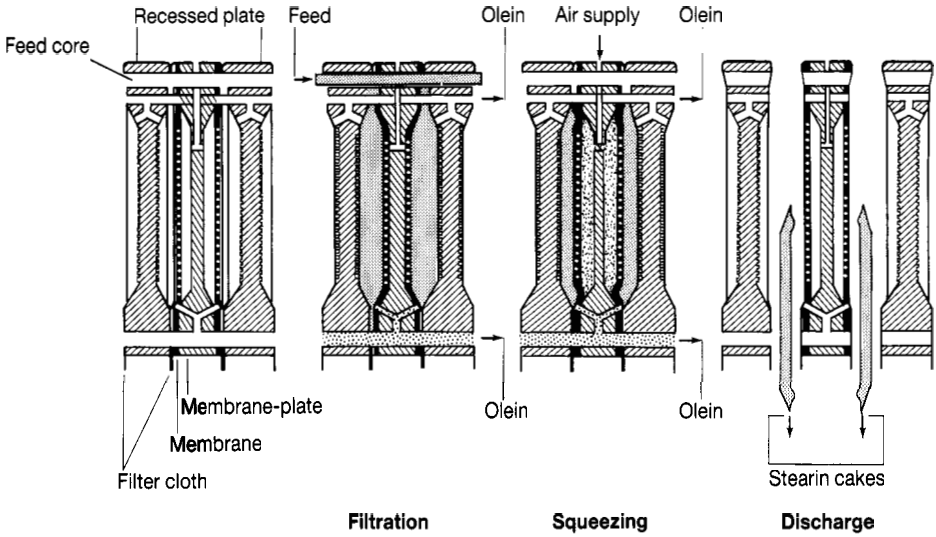


Fig. 8.10. Low-pressure membrane filtration: principle of operation (courtesy of s.a. Fractionnement Tirtiaux).

high degree of elasticity that it can be inflated to stretch across to the opposite side of the chamber without any risk of damage. This is a significant improvement over the earlier polypropylene membranes which were damaged easily when exposed to such extreme stress, i.e. even when the chamber is only half-filled with slurry. Consequently, Hoesch membrane filters are able to operate with only one membrane per chamber.

The operation of the filter press is fully automated. When draining the filter press, the various chambers are automatically opened in succession. Squeezing the stearin slurry causes the fat crystals to cake such that it detaches itself in the form of a solid slab from a specially treated cloth. This is in effect, to some extent, automatic cleaning of the filter cloth. This therefore removes the need to clean the filter cloth after each filter charge, and cleaning can instead be limited to two or three times per week.

The energy consumption for membrane filtration is low compared to vacuum filters. De Smet's figures (Kokken, 1992) give the electricity consumption for the filtration of palm oil containing 50% crystals as 0.2 kW tonne^{-1} for the membrane press and 5 kW tonne^{-1} for the rotary drum filter. This large difference is attributed to the high energy requirement of the vacuum pump in the rotary drum filter. Plonis (1985) reported on a study carried out during the early 1980s comparing the Hoesch filter with a conventional vacuum filter. The evaluation was carried out when a 100 tonne d^{-1} membrane filter was installed in an edible oil refinery in Malaysia. Initially, the refinery used the membrane filter for half its filtration requirement, while retaining the vacuum filter in service for the remaining quantity of feed slurry. This therefore provided the ideal opportunity to compare results of products from the same crystalliser charge (Table 8.4). [Note that the 'cloud point' is the temperature at which the oil begins to cloud, following crystallisation under controlled cooling conditions. It is related to the unsaturation of the oil, and decreases as unsaturation of the oil increases.] A statistical analysis of the results also confirmed that the mean values obtained indicated a high degree of reliability and reproducibility. The olein yield, the iodine value and higher melting point of the stearin were statistically accurate and compatible with the iodine value and cloud points of the olein.

Kokken (1988) reported on the production of super oleins i.e. palm oleins at cloud points below 8°C, as well as on the fractionation of animal and vegetable oils using the membrane filter (Kokken, 1990).

Table 8.4. Comparative fractionation of palm oil using (a) a membrane filter press (Hoesch), and (b) a vacuum filter (Plonis, 1985)

		(a) Membrane filter press	(b) Vacuum filter
Olein	Yield (%)	78.0	66.4
	iodine value	56.95	57.07
	cloud point (°C)	8.87	8.86
Stearin	iodine value	34.32	41.70
	melting point (°C)	53.86	49.99

Factory scale production (100 tonnes/day) using 34 charges from the same crystalliser.

Milk fat fractionation using low pressure membrane filtration has been investigated in several countries including New Zealand, (Table 8.5; Illingworth, 1990) and England (Rajah, 1987), but the first commercial plant was installed in Belgium by De Smet in 1986. Soon after, New Zealand commenced commercial manufacture of fractionated milk fat products using membrane technology. Typical products range, relevant technical data, and their function in various food applications are summarised in Table 8.6. Recently, multi-step fractionation of milk fat has become the subject of much interest and close study (Deffense, 1993). Typical results for oleins recovered from the third step are given in Table 8.7 (Kokken, 1992).

Table 8.5. Milk fat stearins from membrane filtration (Illingworth, 1990)

Separation temperature (°C)	Drop point (°C)	Solid fat content (%)				
		N_0	N_{10}	N_{20}	N_{30}	N_{40}
28	46.6	87.7	84.5	69.5	51.3	23.0
25	44.7	85.7	81.2	61.1	41.0	14.1

High pressure

The process for high-pressure filtration of fat was pioneered by Krupp Maschinentechnik GmbH in the late 1980s, with commercial scale evaluation commencing in 1990 (Willner *et al.*, 1992).

In principle, the Krupp Statofrac® process is descriptive of highly selective fractionation, where an efficient high pressure filter, the Hydrofilter Press (HFP), is used to remove significantly more olein from the fat crystals than has been possible with the already impressive performance of the low-pressure membrane filters. In comparative terms, the Statofrac® process is claimed to be capable of olein yields above 82% while the latter are known to yield up to 80% (compared to 70% yields for the continuous vacuum filters). This process would therefore be of particular interest to those involved in the production of speciality fats such as cocoa butter replacers (CBRs), while due to the variable pressure control facility of the HFP, the process is suitable for application generally to all fats. The evidence for the 1990s therefore points towards the replacement of detergent fractionation, which is used mainly in CBR production, with dry membrane fractionation.

The flow diagram for the Statofrac® process, Fig. 8.11, describes a two-part crystallisation stage, comprising pre-crystallisation and static crystallisation. After melting and precrystallisation in the stirred vessel (B 01), the feedstock is transferred into the maturation section (MS 02), where the crystals grow under slow cooling and low or no (i.e. static) movement. This crystallisation procedure is necessary to ensure the production of crystals which can withstand high-pressure filtration. Such crystals, when squeezed dry, resemble those produced using solvents in terms of their solid fat content (Fig. 8.12; Willner, 1993). The crystal slurry which develops upon maturation is

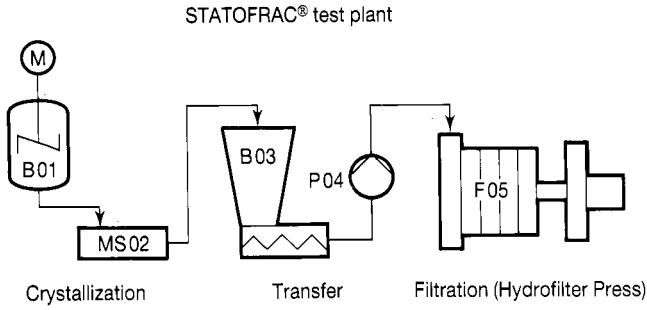


Fig. 8.11. Dry fractionation process combining stirring and static crystallisation for high-pressure press filtration.

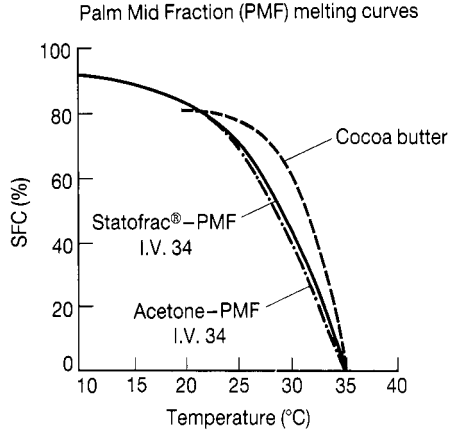


Fig. 8.12. Comparison of melting curves (SFC by NMR according to IUPAC 3.232) of dry fractionated PMF with solvent (acetone) fractionated PMF (literature, data – reference Wong Soon, *A Development Approach to Cocoa Butter Replacers*, Vivar Printing, Kuala Lumpur, 1987) and cocoa butter, with both PMFs having identical iodine values.

transferred into a conditioner (B 03) and passed gently through a conveyor into the Hydrofilter Press (F 05).

Hydrofilter press

The HFP is a membrane filter press, designed for the processing of material such as fat crystals which are sensitive to shearing forces, Figs 8.13 and 8.14. The filtration chambers are circular and this ensures that the membranes are free from stress peaks

Table 8.6. Food applications for milk fat fractions produced using membrane filtration

Product	Melting point (°C)	Form	Solid fat content (%)								Functions in food applications
			N_0	N_5	N_{10}	N_{20}	N_{25}	N_{30}	N_{35}	N_{40}	
1. Soft butteroil	20–22	Liquid olein	51–58		30–37	2–8					Surface spraying of bakery products, e.g. bread to promote contraction, crust development and gloss.
2. Soft butteroil	27–29	Liquid or in plasticised form		56–60	49–53	12–16	2–5				Flavour and stable emulsions in ice cream and recombined dairy products.
3. Cookie butter	27–29	Plasticised		56–60	49–53	12–16	2–5				In shortbread and cookies to overcome fat bloom. In cakes for flavour and light texture
4. Butter-shortening	36–38	Plasticised blend of milk fractions			49–52	25–30	17–22	9–14	4–7		Creaming shortening, i.e. aeration for cakes and buttercreams. Also for use in Danish pastries for flavour and development of open texture.

Table 8.6. (*continued*)

Product	Melting point (°C)	Form	Solid fat content (%)								Functions in food applications
			N_0	N_5	N_{10}	N_{20}	N_{25}	N_{30}	N_{35}	N_{40}	
5. Bakery butter	36–38	Plasticised blend of fractions and fresh cream			54–62	24–31	16–22	8–14	3–7		Replaces butter in high-quality bakery applications, including buttercream fillings
6. Confectionery butterfat 42	41–43	Plasticised high melting stearin	74–80		70–76	46–54		24–34		4–11	Forms higher solid fat content in blends with cocoa butter than is possible with standard milk fat. Also, anti-bloom effect in chocolate. Other confectionery includes caramel fudges and fondant.

Table 8.7. Three-step fractionation of milk fat using membrane filtration, and typical results on the olein of the third step (Kokken, 1991)

Yield	20–25%
Iodine	47–48%
Cloud point	0.5–1.0°C
Solid fat content (SFC)	
0°C	15–18%
5°C	5–8%
10°C	0

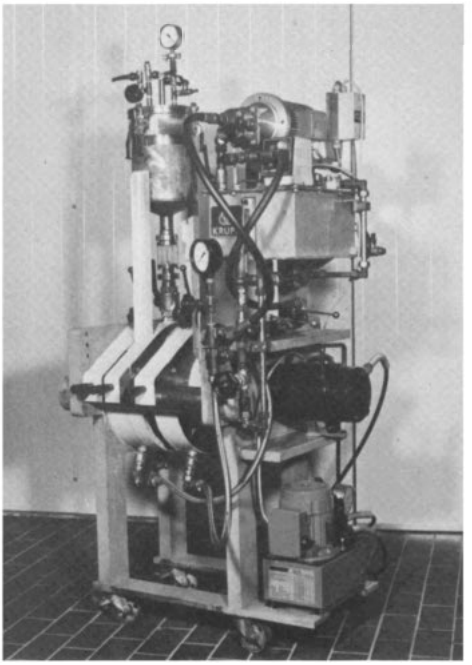


Fig. 8.13. Laboratory scale pilot plant: Hydro Filter Press HFP03 (courtesy of Krupp, Germany).

normally associated with designs which have corners. This is important because the applied pressure can reach up to 50 bar.

Each press has several chambers, and a block of chamber plates is enclosed and held together by means of a hydraulic closing unit.

The crystal slurry or suspension is filled into each chamber, and when full the chamber feed line is closed. On the front facing side of each chamber is a filter sieve which is plane and made of stainless steel, of very fine mesh, held vertical for good cake release when the chambers are opened after filtration. Opposite, facing the rear, a flexible

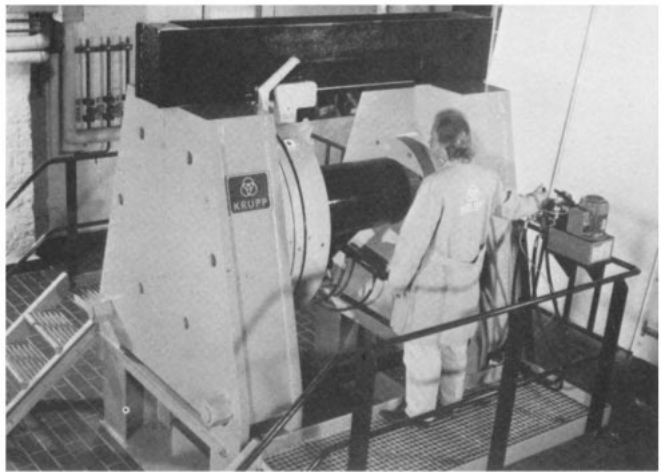


Fig. 8.14. Pilot plant: Hydro Filter Press HFP04 (courtesy of Krupp, Germany).

mpermeable membrane is attached to surround and cover that side completely. When filtration commences, the suspension is pressed against the filter sieve by the flexible membrane. The filtrate (olein) passes through the sieve and drains to the bottom outside the press where it is collected. The crystals (stearin) remain within the chamber and are pressed into a cake, the press cake, as the olein is squeezed out.

The filtration pressure exerted by the membrane is generated by the hydraulic liquid. The application of a liquid in preference to a gas reduces the risk of explosions when working at very high pressures. It is feasible to use the filtrate as the hydraulic liquid, the advantage being that in case of membrane damage the fractionated products are not contaminated with inedible or toxic material.

During the filtration cycle, the filtration pressure is increased slowly up to the end pressure, a recommended maximum of 50 bar. The rate of pressure increase is programmable and the whole filtration process is automated. The design of a 25 tonnes d^{-1} hydrofilter press is shown in Fig. 8.15.

The fractionation of palm kernel oil and palm oil has been investigated in detail, and some work has also been completed on milk fat (butterfat). Some of the results are reported here (Willner, 1993).

Figures 8.16 (a, b) show the schematic and process conditions for the production of palm kernel stearins, particularly PKS IV 7 suitable for hardening into cocoa butter substitute (CBS) manufacture.

The Statofrac® process is also shown to be suitable for the production of palm mid-fraction (PMF) which is used in the manufacture of cocoa butter equivalents (CBEs) (Fig. 8.17 (a–c)).

The yield of palm olein increases with filtration pressure (Fig. 8.18), and this correlates with the reduction in iodine value of the stearin fraction.

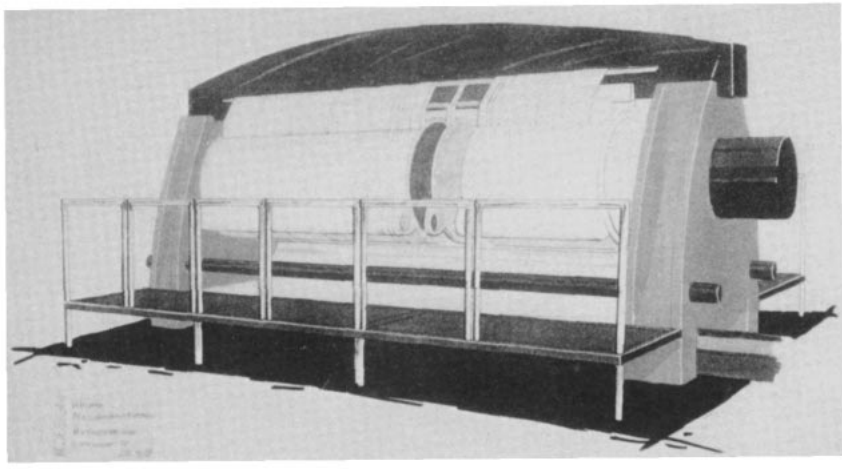


Fig. 8.15. A production scale 25 tonnes d^{-1} Hydro Filter Press HFP02 (courtesy of Krupp, Germany).

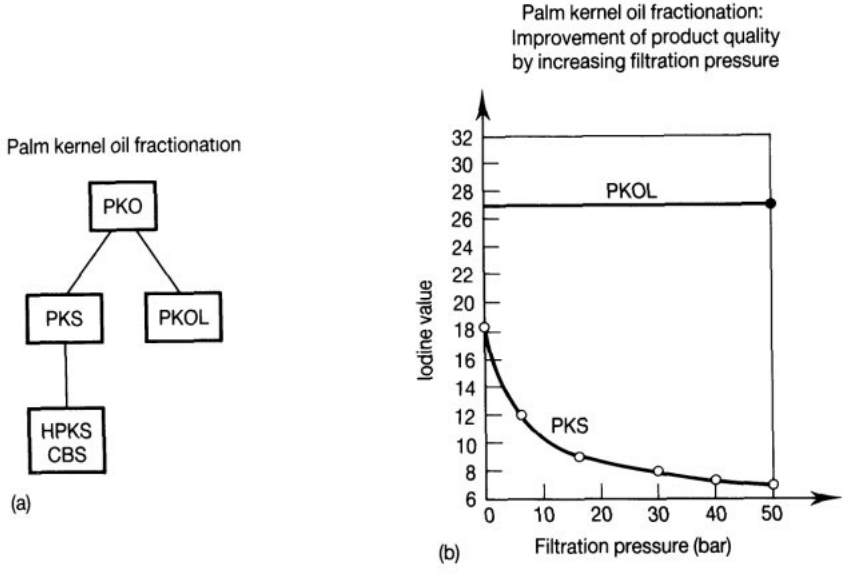


Fig. 8.16. (a) Single-stage dry fractionation of palm kernel oil for the production of PKS IV 7 suitable for hardening for high-quality CBS production; (b) increase in pressure during HFP filtration squeezes out more olein from the crystals, resulting in the production of very low IV stearins.

The fractionation of milk fat yields stearins of solid fat content (SFC) values in the region of $N_{10} = 85\%$ (Figs. 8.19 (a, b)). This is indicative of more efficient removal of the olein phase from the crystals than those reported for vacuum filtration by Ricci-Rossi and Deffense (1984).

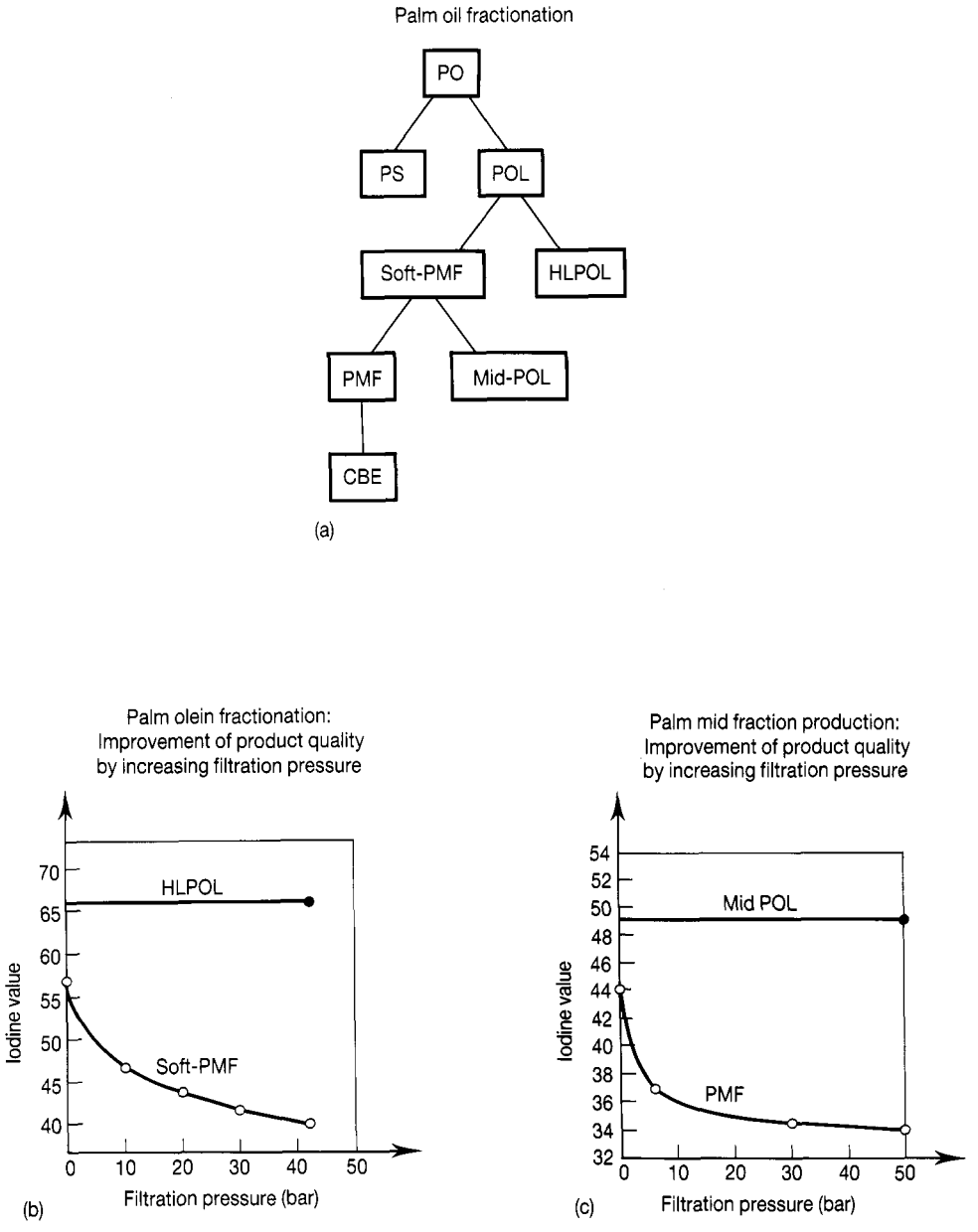


Fig. 8.17. (a) Three-stage dry fractionation of palm oil for the production of CBE grade PMF and highly cold-stable olein fractions HLPOL IV68 and MidPOL IV 49; (b) fractionation of the POL suspension and filtration using the Hydro Filter Press for the production of low IV, soft-PMF and high-IV HLPOL; (c) Hydro Filter Press filtration of the soft-PMF suspension for the production of CBE-grade PMF.

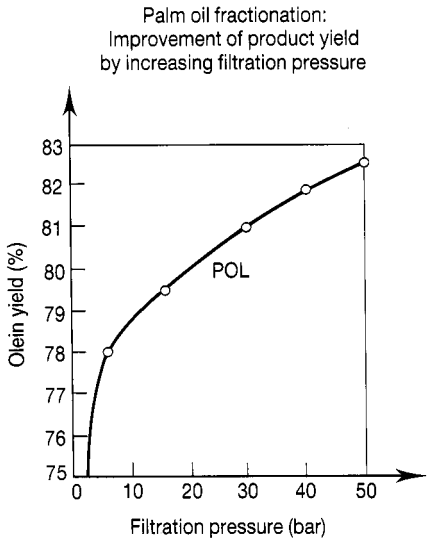


Fig. 8.18. High-pressure filtration, using the Hydro Filter Press, increases the olein yield.

8.3 DETERGENT FRACTIONATION

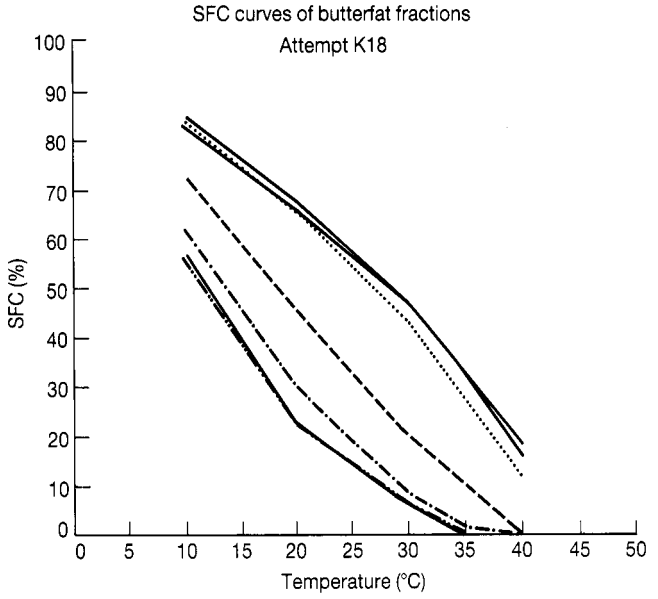
8.3.1 The Lipofrac process

The principles of the detergent process were first described by Fratelli Lanza in 1905 and it consequently also became known as the Lanza process. More recently Alfa Laval (now Tetra-Laval, based in Tumba, Sweden) developed a commercial process (Fjaervoll, 1969 and 1970) and called it Lipofrac fractionation. In principle, fractional crystallisation (Saxer and Fischer, 1983) forms the first stage. When the crystals have formed, water containing an aqueous detergent (sodium lauryl sulphate) and an electrolyte (magnesium sulphate or sodium sulphate) is added and the crystals become dispersed in it; the electrolyte facilitates the agglomeration of the oil droplets in the succeeding mixing process. Separation of the crystals is completed by centrifugation.

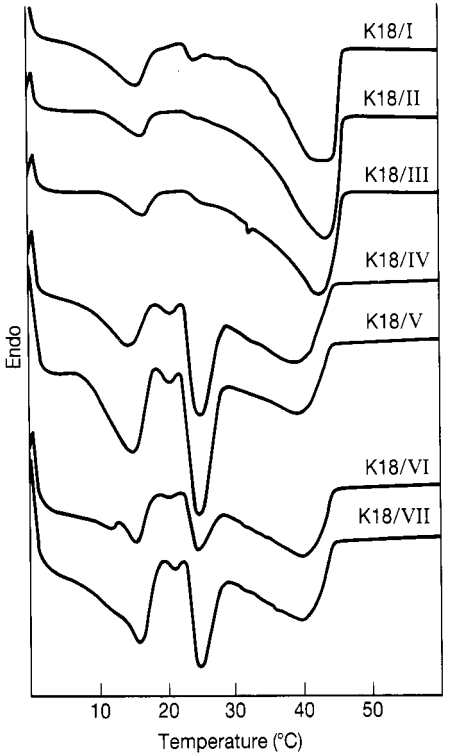
8.3.2 Crystallisation

Since the Lipofrac process was first introduced a number of improvements have been made to increase the yield of olein and to maximise on plant efficiency and capacity. The most notable improvement was made in the mid-1980s when a substantially modified procedure for fat crystallisation was introduced (Bauren, 1986). Until then crystallisation of fat was being carried out in 14 or 28 m^3 batch crystallisers fitted with agitators capable of scraping the walls, the walls also forming the cooling surface for the crystalliser. This low efficiency design combined low agitation rates with a limited low-temperature surface area and low-temperature water-cooling regimes.

Alfa Laval modified this operation and achieved shorter crystallisation times while making the whole process continuous (Fig. 8.20). The cooling of the fat was transferred



(a)



(b)

Fig. 8.19. (a) A selection of SCF curves on butterfat stearins obtained from high-pressure dry fractionation (SFC by NMR according to DGF); (b) DSC curves of corresponding stearin fraction in (a) (Willner, 1993).

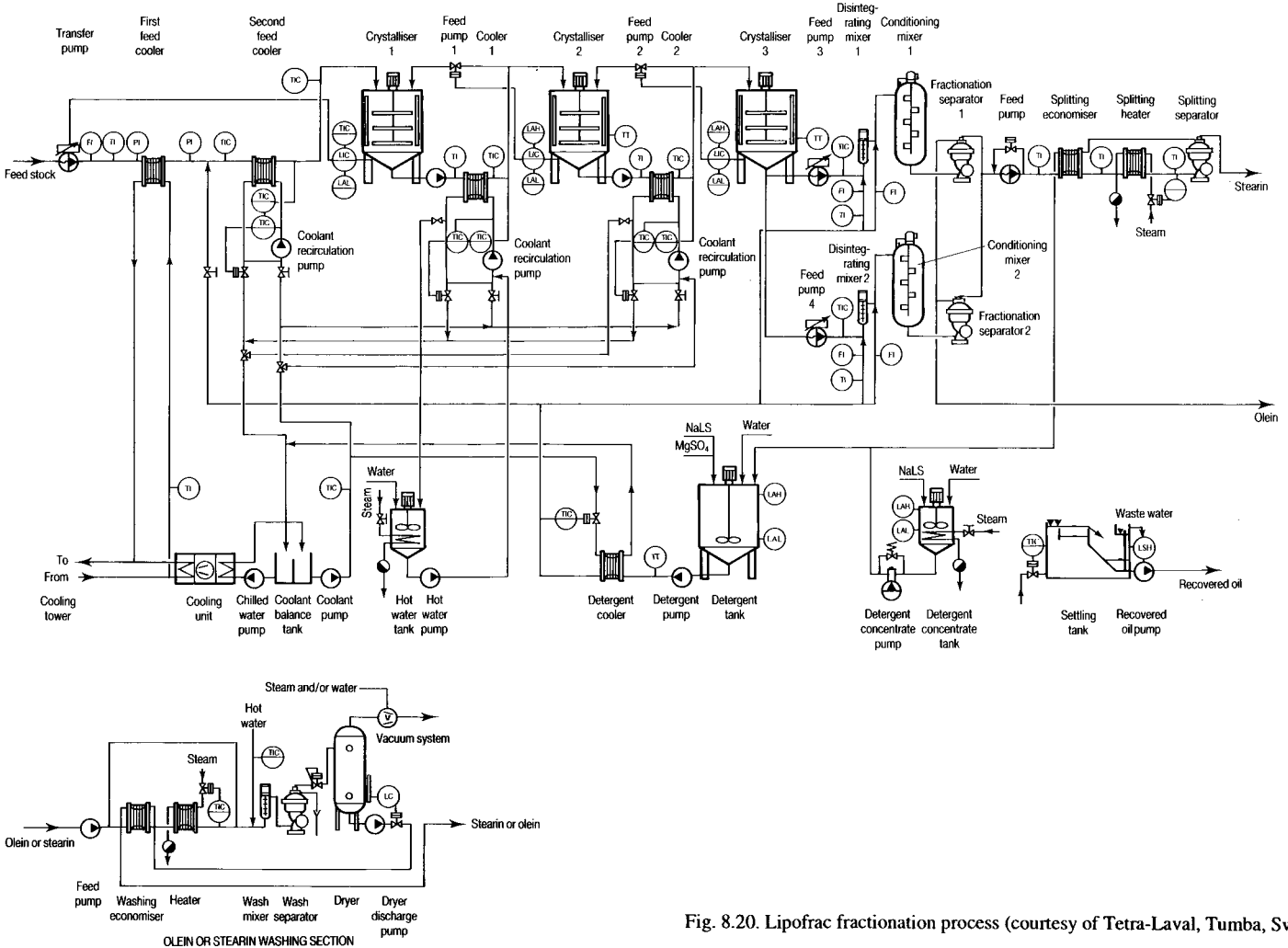


Fig. 8.20. Lipofrac fractionation process (courtesy of Tetra-Laval, Tumba, Sweden).

to efficient external plate heat exchangers, thus making it possible to use inexpensively constructed tanks for the required holding time.

For palm oil fractionation, crude palm oil from the storage tank is first cooled, in precooler 1, to 35°C, using water from a cooling tower as coolant. Some detergent is then usually added before the second precooler where the crude palm oil is cooled further to between 25–27°C, again by water cooling. At this point the oil is transferred to crystalliser 1 for a holding time of about 30 min. No cooling takes place in the crystalliser but some increase in temperature follows due to the heat of crystallisation. The mixture, comprising liquid oil, crystals and detergent, is pumped over to crystalliser 2 through a plate heat exchanger which cools it by a further 3–5°C. After holding the mixture in crystalliser 2 for a time it is pumped into crystalliser 3 through the second heat exchanger, which cools it down to the final fractionation temperature or lower. After a further holding time the mixture is ready for separation. An additional crystalliser and a third heat exchanger would be required for feed quantities exceeding 300 tonnes per 24 h.

The temperature of the cooling water circulating in the plate heat exchangers is in the region of 3–10°C below the temperature of the oil. To avoid fat crystallising on the contact surfaces the temperature of the coolant has to be controlled closely. Nevertheless some crystallisation does take place, particularly in the corner areas of the plate heat exchangers where the flow is very slow moving or stagnant. When this happens the oil flow is diverted to by-pass the heat exchanger and hot water is introduced into the circulation to melt any crystals.

It is reported (Bauren, 1986) that the revised crystallisation system enables uniform cooling of the whole feedstock, thus ensuring consistent quality feed slurry for separation. It is also maintained that crystal size distribution is narrower than those achieved on the earlier crystallisers and that the average crystal size is also larger, which therefore reduces the amount of wetting agent required. The energy losses from the plate heat exchangers are also less than those suffered from the earlier crystallisers.

The scheme described above is designed for the crystallisation of palm oil for the production of RBD (refined, bleached and deodorised) palm olein with a cloud point of 8°C. The system is, however, flexible, and with some minor modifications the same equipment can be used for other raw materials, e.g. fatty acids, palm kernel oil etc.

Separation of the mixture is preceded by the addition of more detergent solution and heavy mechanical working to disintegrate any crystal agglomerates which could trap liquid oil and thereby prevent the wetting of some of the crystals. Even more addition of detergent follows and the mixture is then held under gentle agitation to enable the oil droplets to form a continuous phase. When ready, the mixture is fed to the centrifugal separator, where the olein is removed as the light phase and the suspension of crystals in the detergent solution is the heavy phase. The crystal suspension is then heated to melt the fat crystals, the stearin, which then becomes the light phase and is removed in a second separator. The detergent solution, now the heavy phase, is returned to the detergent tank and reused. It is reported that 8–10 ppm of surface active agent remains in the fractions (Bernadini, 1973). The yield of palm olein from Lipofrac fractionation is usually about 80% at iodine values of 52, 57 and 30 for crude palm oil, palm olein, and palm stearin respectively. The critical factor determining the yield is the amount of olein remaining in the stearin suspension as droplets. These droplets are so fine that they are

not separated as part of the olein phase. If the formation of small droplets can be reduced, the yield can be increased. To get a measure of the potential theoretical increase in yield laboratory studies were carried out on palm oil (Bauren, 1986). Palm stearin suspensions from three pilot plant fractionations were first washed to remove all remaining olein. The iodine values (IV) were determined and found to be 26.7, 23.8 and 19.7. The spread was rather wide, attributed mainly to difficulties in sampling and analysis, but it was nevertheless possible to conclude that the maximum possible yield should be somewhat better than 85%.

The separation of saturated and unsaturated fatty acids has been discussed by Haraldsson (1984). He also earlier (1978) reviewed the production of hard butters from palm oil fats. Another typical process employing aqueous detergent fractionation is the Henkel process (Stein and Hartman, 1957). Jebson and Lochore (1975) compared the efficiency of the Henkel filtering centrifuge with that of the Alfa Laval equipment for the fractionation of milk fat. The Henkel centrifugal filter, normally a punched or wire mesh screen mounted on a rotating cone, yielded a maximum of 60% crystals, compared with 70% using the Alfa Laval process, on fat crystallised at 25°C.

The New Zealand Dairy Research Institute (Norris *et al.*, 1971) published a report on the fractionation of milk fat using a detergent process. However, due to world-wide resistance from those within the industry and consumers alike against any treatment of milk fat with additives even if only as a processing aid, such processes have never been scaled up to industrial manufacture. More recently Glassner (1987) studied the separation mechanism for the detergent fractionation of beef tallow and its relation to key process variables. Crystallisation conditions, the amount of surfactant (SDS), the electrolyte (sodium citrate) concentration, the weight ratio of detergent solution to partially crystallised tallow and the viscosity of the dispersion all formed important process considerations. He cited the example of beef tallow separated at 40°C, where all these variables affected the level of separation. He found, for instance, that over-dosing with surfactant caused the olein to emulsify while too low an inclusion resulted in incomplete separation. Apparently the selection of the correct dispersion had a significant effect in maximising the olein yield (Glassner and Grulke, 1987).

8.4 SOLVENT FRACTIONATION

During solvent-aided fractionation either apolar (hexane) or polar (isopropyl alcohol, acetone or 2-nitropropane) solvents may be used. Typically, during commercial fractionation using the Bernadini process, hexane is used in a 1:1 proportion for palm-oil fractionation. Alternatively, in a Unilever patented process, 3 parts of acetone are mixed with 1 part of palm oil. In the HLS process, the polar isopropyl alcohol is mixed with palm oil in a 1:1 ratio (Hoffman, 1989). Fractionation by solvents is important when fats (containing a high proportion of triacylglycerols comprising long-chain fatty acids) remain highly viscous or even solid at temperatures normally associated with fractionation. It is based on the underlying principle that fractional crystallisation from dilute solutions is more efficient with respect to separations than when solvents are not used. Consequently yields of olein are higher and stearin purity is much improved, while processing time is also reduced. Although these benefits are significant they are partially

off-set by the relative high cost of the initial capital investment as well as the high operational costs involved, particularly in relation to energy requirements and solvent loss estimated at 3–10 kg tonne^{-1}. During solvent fractionation (Fig. 8.21) it is necessary to cool not only the oil, but the entire quantity of solvent as well, which adds to the cost. Three major solvent processes are understood to be in commercial operation. These are the Bernadini CMB process (Bernadini and Bernadini, 1971, 1975; Kreulen, 1976), the Emersol process (Muckerheide, 1960; Zilch, 1967) and the Zondek process (Zondek, 1978). Other processes are also available and have been reviewed (Thomas III, 1985). Most solvent processes can also be used for winterisation of oils.

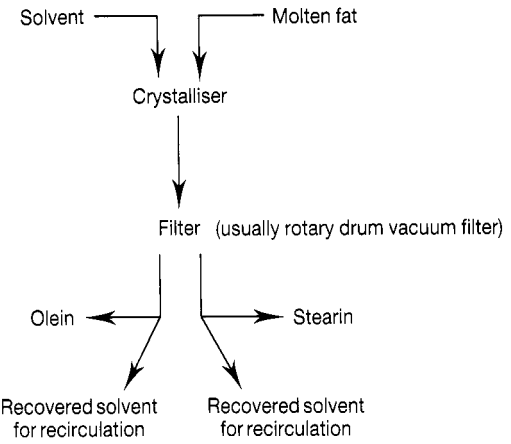


Fig. 8.21. Schematic diagram describing the general principles of solvent fractionation.

The crystallisation temperatures are in the range from 20°C down to 0°C or less (Rossell, 1985). Filtration is often through a rotary drum filter which is enclosed to prevent solvent loss, Fig. 8.22.

Jebsen *et al.* (1975) have outlined a process for solvent fractionation of milk fat using a batch plant designed to process one tonne of milk fat per day. It is reported that to produce good quality stearins, i.e. with a minimum amount of the liquid phase entrapped in the crystal structure, lower coolant temperature and faster stirring rates were critical factors (Munro *et al.*, 1976). However, flavour problems from solvent residues in fractional products make solvent fractionation of milk fat impracticable (Cant *et al.*, 1975).

The use of carbon dioxide as a solvent in supercritical extraction (SCE) of fat fractions is a relatively recent process. This is discussed more comprehensively in Chapter 2. It is understood that the most recent activity in this area is being undertaken at the University of Wisconsin, where SCE is used to fractionate milk fat and, in a second stage process, the fractions are then modified using lipase enzymes to yield specific end-product fractions for a variety of food uses (Dairy Foods, 1989).

Solvent fractionation is now being challenged by high pressure membrane filtration. The evidence to date suggests that the quality of the fractions produced by the latter is

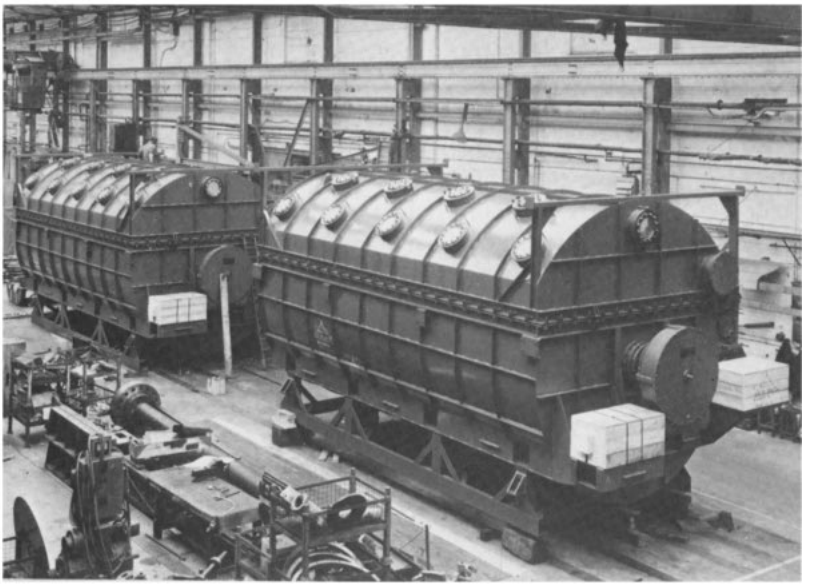


Fig. 8.22. Rotary vacuum filter fully enclosed for use in solvent fractionation (courtesy of Filtration Services Ltd, Macclesfield, UK).

similar to that from the former. In addition, issues concerning safety and costs, both initial capital outlay and running costs, make high-pressure dry fractionation a viable option.

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Chapter 9

Solids separation processes

M. J. LEWIS, Department of Food Science and Technology, The University of Reading, RG6 6AP

9.1 INTRODUCTION

This chapter will cover the separations involving solid foods, together with the properties of those solids which will influence that separation. Some mention will also be made of handling and transporting solids and preparatory processes, such as size reduction. The separation of solids from liquids and solids from gases is not covered in detail in this chapter, although a summary of the methods based on sedimentation and filtration is given in Table 9.1. In these applications, the term solids refers to discrete particles suspended within the fluid and not those dissolved or in the colloidal form, for which a range of other operations for their removal or separation is available. The objective may be to recover the solid for further processing or to remove the solid which may be contaminating the liquid or gas. The method selected also depends upon whether the solid is to be retained or discarded.

To illustrate some of the difficulties in selecting solids separation methods, the removal of solids from gases will be further illustrated. This can be achieved by classifiers, cyclones, bag filters or electrostatic precipitators. In cyclones on milk powder plant, particles less than 5–10 μm may be lost. Cyclone losses of 0.35–1.0% of total production have been cited for dairy products. Such losses are now unacceptable for environmental reasons. High-efficiency cyclones have been used, whereby secondary air is introduced into the cyclone to increase the efficiency. However, these cyclones are not very successful with powders containing fat, as considerable free fat is generated and the powder sticks to the interior surface of the drier. Therefore it is not possible to install a milk drier where the powder recovery system consists of cyclones alone. Wet systems such as scrubbers have been installed, using the pasteurised milk, prior to evaporation, as the scrubbing liquid, thereby recovering the fines and heat. From a recovery standpoint, this would seem an excellent solution. However, from a hygiene and quality standpoint, these proved almost impossible to operate without bacteriological contamination. Most of these have now been removed (Knipschildt, 1986). The solution to the problem has been provided by bag filters, which are capable of reducing the particle concentration from

Table 9.1. Summary of mechanical solid separation techniques

Solids from liquids
Sedimentation:
Principles: gravity, centrifugal, electrostatic, magnetic centrifugation
Examples: gravity settlers, centrifugal clarifiers, hydrocyclones; use of chemical flocculants or air flotation
Filtration: (see also Chapter 8; fat fractionation)
Principles: gravity, vacuum, pressure and centrifugal
Examples: sand and cake filters, rotary vacuum filters, cartridge and plate and frame filters, microfilters (Chapter 5); use of filter aids
Solids from gases
Principles: sedimentation and filtration
Examples: cyclones, bag filters, electrostatic precipitators

200 mg m^{-3} to below 10 mg m^{-3} air. The powder can be recovered from the bags and the 'clean air' can be used for heat exchange. Further details are provided by Knipschildt (1986).

However, rather than removing all the particles, there may be a requirement to fractionate the powder, based on particle size (see Sections 9.3 and 9.4). This example illustrates the theme for this chapter, where the main emphasis is placed on the separation of components from within a solid matrix. Solids come in many forms, shapes and sizes, so the first part of the chapter will be devoted to discussion of the main properties of solid foods which will influence the different types of separation processes.

9.2 PHYSICAL PROPERTIES OF SOLIDS

Solids come in a wide variety of shapes and sizes. All solid foods are particulate in nature and there are a wide range of sizes and shapes to contend with. Some examples are illustrated from the different food sectors in Table 9.2. It should be noted that although all these foods are regarded as solids, their moisture content may range from less than 10% to greater than 90%. Their moisture content and chemical composition can be found from foods composition tables, for example Paul and Southgate (1978) (see also Chapter 2). Indeed, one of the main objectives is often to remove selected components from the food.

Some operations where separations from solids is involved and constitutes an important part of the process are:

- cleaning of agricultural produce (see Section 9.6.3);
- sorting and size grading, particularly for quality grading of fruit and vegetables;
- peeling of vegetables, dehulling of cereals and legumes and deboning or shelling of meat and fish;
- fractionation or recovery of the main components within the foods, e.g. proteins, fat, carbohydrates and minerals.

Table 9.2. Some examples of solid foods

Fruit: apples, oranges, grapes, blackcurrants, pears, bananas
Vegetables: potatoes, carrots, sprouts, peas
Cereals and legumes: rice, wheat, soyabeans, cowpeas, sorghum
Animal produce: large carcasses, small joints, minced meats, fish fillets, prawns, shrimps and other shellfish
Beverages: coffee beans, tea leaves, instant powders and granules
Other powders: milled products, powders produced by drying and grinding methods

A special range of operations and an area of increasing interest is concerned with the separation or fractionation of solids, in their particulate or powder form, and their recovery from other materials. In this chapter, emphasis will be placed on the separation of powders, based on factors such as size and shape, density differences, flow properties, colour and electrostatic charge. An important pretreatment for many such operations is size reduction. Methods of size reduction are discussed in Section 9.3.1. Size reduction increases the surface area and the surface area to volume ratio, thereby enhancing rates of heat and mass transfer.

However, in some cases very fine powders provide processing problems, and size enlargement or agglomeration may be used to improve flow characteristics and wettability.

Many foods which are solid in appearance, will also flow if the shear force provided is great enough, e.g. butter, spreads and starch doughs. This behaviour is known as plasticity. The flow behaviour of powders is also important and is discussed in more detail in Section 9.2.7. Some of the important physical properties of solid foods are listed in Table 9.3. These are discussed in more detail by Lewis (1990), Jowitt *et al.* (1983, 1987), Mohsenin (1984, 1986) and Peleg and Bagley (1983). Many of these properties are influenced by the chemical composition of the food, and in particular its moisture content.

Of special interest in this context is the behaviour of particulate systems and the separation of mixtures. Many such separations are based on density differences. In some cases the powders may be subjected to various forces, gravitational, which are slow

Table 9.3. Physical properties of solids

Appearance, size, shape, size distribution, colour
Specific gravity, particle density, bulk density, porosity, overrun (for aerated products)
Thermal properties; specific heat, latent heat, thermal conductivity, thermal diffusivity, specific enthalpy
Rheologial properties; plasticity, elasticity, viscoelasticity, hardness
Electrical conductance or resistance, electrical charge, dielectric constant, dielectric loss factor
Diffusion and mass transfer characteristics

compared to centrifugal forces, drag forces or electrical, electrostatic or magnetic forces. Also, the flow characteristics and behaviour of food powders are markedly different to those of fluids.

Some of the physical properties of food powders will now be considered in more detail, especially those which will influence the effectiveness, quality and nature of the separation process.

9.2.1 Classification of powders

Powders can be characterised in a large number of ways; Peleg (1983) gives some examples:

- by usage: e.g. flours, beverages, spices, sweeteners;
- by major component: e.g. starchy, proteinaceous, fatty;
- by process: e.g. ground powders, freeze-dried, agglomerated;
- by size: e.g. fine, coarse;
- by moisture sorption characteristics: e.g. hygroscopic;
- by flowability: free flowing, sticky, very cohesive.

Further classification could be by hardness, by explosion potential or by microbial hazards. Hayes (1987) summarises a detailed system used for characterising a wide range of food powders based on density, size, flowability, abrasiveness, a range of miscellaneous properties and hazards such as flammability, explosiveness and corrosive nature.

Some important physical, chemical and functional properties of powders are given in Table 9.4. For products such as beverages, the palatability and sensory characteristics of the reconstituted products are important and may be variables considered when grading these products. Care should also be taken to ensure that the microbial count is within acceptable limits for the products.

Determination of some of these properties for milk powders is described in publications by the Society of Dairy Technology (SDT, 1980), and Schubert (1987a).

Table 9.4. Factors contributing to the quality of powders

Appearance
Size and shape
Wettability
Sinkability
Solubility
Dispersibility
Bulk density and particle density
Palatability
Nutrient content
Microbiological quality

9.2.2 Particle size and particle size distribution

As mentioned earlier, food powders come in a wide range of sizes and shapes. Uniform shapes, such as spheres, can be characterised by one dimension, i.e. the diameter, whereas two or more measurements may be required for more complex shapes. Whatever the shape, there are several methods available to characterise the size and particle size distribution. Virtually all operations that result in the production of a powder, e.g. milling or spray drying, will give rise to a product with a distribution of particle sizes and this distribution is of extreme importance and will affect the bulk properties. Particle size may range over several orders of magnitude, ranging from less than 1 μm to as large as hundreds or even thousands of microns for some large granules. Particle size can be measured in principle by measuring any physical property which correlates with the geometric dimensions of the sample. According to Schubert (1987a) the attributes used to characterise particles may be classified as follows:

- geometric characteristics, such as linear dimensions, areas or volumes;
- mass;
- settling rates;
- interference techniques such as electrical field interference and light or laser scattering or diffraction.

Based on these attributes, the following methods have been used for food materials:

- microscopy or other image scanning techniques;
- wet and dry sieving methods;
- electrical impedance methods such as the Coulter counter;
- laser diffraction patterns, such as the Malvern, Northrup and Cilas instruments.

Since particles can vary in both shape and size, different methods of particle size analysis do not always give consistent results, both because of the different physical principles being exploited, but also because size and shape are interrelated. Sampling is also important to ensure that a representative sample is taken, usually by the method of quartering.

Whatever method of measurement is used, a large number of particles must be measured in order to ascertain the particle size distribution. It has been suggested for light microscopy that 200 measurements are made on each of three separate slides (Cloutt, 1983); this makes the method very tedious. The simplest way to present such results is in the form of a distribution curve, the two most common being in the form of either a *frequency distribution* (histogram) or a *cumulative distribution* (see Fig. 9.1). The cumulative distribution can be based on percentage oversize or percentage undersize. Percentage undersize is used more often. The method used for data collection may give a distribution in terms of number of particles (for example by counting) or the mass (weight) of particles (for example by sieving).

If the number of particles is known, the distribution can be represented by a frequency distribution. Table 9.5 gives some typical figures for the number of particles collected by microscopical examination, arranged into numbers falling within different size ranges (0–10 μm) etc., together with the frequency distribution and cumulative number distribution undersize.

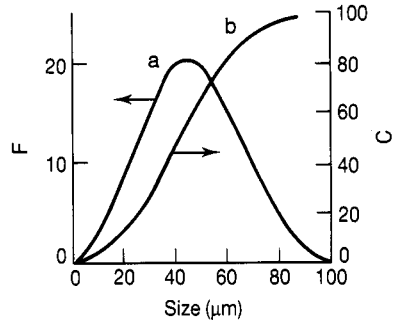


Fig. 9.1. (a) Frequency distribution (*F*), (b) cumulative distribution (*C*): see also data in Table 9.5.

Table 9.5. Frequency distribution

Size range (μm)	Mean diameter of range (μm)	Number in range	Frequency distribution	Cumulative distribution	Cumulative volume distribution
0 to 10	5	5	1.8	1.8	0
10 to 20	15	15	5.6	7.4	0
20 to 30	25	35	12.9	20.3	2.0
30 to 40	35	50	18.4	38.7	7.0
40 to 50	45	55	20.3	59.0	20.0
50 to 60	55	50	18.5	77.5	41.0
60 to 70	65	32	11.8	89.3	64.0
70 to 80	75	20	7.4	96.7	85.0
80 to 90	85	8	2.9	99.6	98.0
90 to 100	95	1	0.4	100.0	100.0
>100		0			
		271			

mean diameter = 45.96 μm; $d_{2/1}$ = 53.29 μm; $d_{3/2}$ = 58.86 μm

Cumulative number frequency indicates the percentage of the total number less than the mean diameter of the range.
Cumulative volume represents the percentage of the total volume less than the mean diameter of the range.

Other values which may be calculated from the distribution include the mean diameter and the median diameter and the standard deviation, which gives an indication of the spread.

The simplest is the mean diameter, defined as

$$\sum n_i d_i / \sum n_i ,$$

where n_i is the number of particles in class i and d_i is the mean diameter of class i. The median diameter is the diameter which cuts the cumulative distribution in half. The $d_{2/1}$ ratios and $d_{3/2}$ ratios are also calculated.

However, one widely used characteristic is the Sauter mean particle diameter ($d_{3/2}$). This is calculated from

$$d_{3/2} = \sum n_i d_i^3 / \sum n_i d_i^2 \tag{9.1}$$

This gives the diameter of the particle having the same surface area to volume ratio as the entire dispersion.

$$\text{The surface area/volume ratio} = 6/d_{3/2}. \tag{9.2}$$

Rates of heat transfer and mass transfer are proportional to the surface area to volume ratio. Therefore the surface area exposed has a big influence on physical properties, e.g. wettability, dispersion, dissolution and chemical reactions, such as oxidation, as well as the forces acting at the surface of powders. Equation (9.2) demonstrates that decreasing $d_{3/2}$ will increase the surface area to volume ratio.

Such data can be converted to frequency or cumulative distribution based on surface area or volume, by calculating the surface area and volume of each range. These cumulative distributions based on numbers and volume are compared in Fig. 9.2. This distinction is made because the shape of a numbers distribution and a mass or volume distribution is quite different because the area and volume distributions are most influenced by the larger diameter particles, since the volume = $\frac{4}{3}\pi r^3$. For example, it can be seen that only 10.7% of the particles are greater than 65 μm, whereas on a volume basis, 36% by volume are greater than 65 μm (Fig. 9.2). The weight fraction distribution would be similar to the volume fraction distribution, provided that the solid density is independent of particle size. The volume distribution is a common form of presentation in emulsion science, since it is often the larger particles which are likely to cause separation problems. Therefore it can be very informative to know what fractions by volume are bigger than a particular size. For example, in cream separation in milk, problems may arise from a relatively small number of large fat globules.

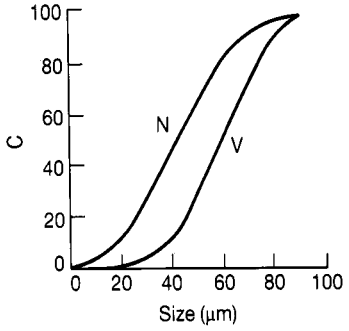


Fig. 9.2. Comparison of volume distribution (*V*) and cumulative number distribution (*N*). See also data in Table 9.5.

Most of the discussion has focused upon spherical particles or those closely approximating to these. However, the particle shape is also very likely to be important and a wide variety of shapes are also found. Irregular-shaped objects are more complicated to define and a number of characteristic dimensions have been used to represent them. Some are given in Table 9.6.

Table 9.6. Characteristic diameters for irregular shaped particles

d_s	Surface diameter	The diameter of a sphere having the same surface area as the particle
d_v	Volume diameter	The diameter of a sphere having the same volume as the particle
d_d	Drag diameter	The diameter of the particle having the same resistance to motion as the particle in a fluid of the same density and viscosity
d_s	Sieve diameter	The width of the minimum square aperture through which the particle will pass.

Other dimensions include the free-falling diameter and Stokes diameter, the projected area diameter and the specific surface diameter. In many cases the shape is more complex and a large number of dimensions would be required to describe the size and shape. Image analysis methods, whereby an image of the object is transferred to a computer screen and software is available to do any number of manipulations and calculations on the shape, are useful for this.

The particle size and distribution has a pronounced effect on interparticle adhesion, which will affect some of the bulk properties, such as bulk density, porosity, flowability and wettability (see Section 9.2.5).

9.2.3 Particle density

The density of an individual particle is important as it will determine whether the component will float or sink in water or any other solvent; the particle may or may not contain air. It can be measured using a specific gravity bottle, using a fluid in which it will not dissolve. Alternatively, it may be measured by flotation principles. However, surface forces may start to predominate for fine powders.

In the absence of air, the particle density can be estimated from the following equation, based on the mass fractions and densities of the food components.

$$\rho = 1/\left[\left(M_1/\rho_1 + M_2/\rho_2 + \ldots + M_n/\rho_n\right)\right] \tag{9.3}$$

where M_1 is the mass fraction of component 1, ρ_1 is the density of component 1 and n is the number of components. Data on mass fractions can be found from the Composition of

Foods Tables (Paul and Southgate, 1978). A simple two-component model can be used ($n = 2$; water and solids) or a multicomponent system.

The density of the major components are given as (kg m^{-3}) (Peleg, 1983):

water	1000	salt	2160
fat	900–950	citric acid	1540
protein	1400	cellulose	1270–1610
sucrose	1590	starch	1500
glucose	1560		

It is noteworthy that all solid components except fat are substantially more dense than water. However the differences between protein and the various types of carbohydrates are less marked, although minerals are much higher. In comparison air has a density of 1.27 kg m^{-3}.

This equation is not applicable where there is a substantial volume fraction of air in the particle. Any deviation between the experimentally determined value and the value calculated from the above equation may mean that there is substantial air within the solid. An estimate of the volume fraction of air (V_a) can be made from

$$\rho = V_a\rho_a + V_s\rho_s = V_a\rho_a + (1 - V_a)\rho_s \tag{9.4}$$

where ρ_a = density of air; ρ_s = density of solid (estimated using eq. (9.2)) and ρ = true solid density, measured experimentally. This volume fraction (V_a) of air is sometimes known as the internal porosity.

Many other foods contain substantial amounts of air, for example mechanically worked doughs. One solution to determine the unaerated density is to measure the dough density at different pressures and extrapolate back to zero pressure (absolute) to obtain the unaerated density. This methodology could then be used to determine the extent of aeration during the mixing process.

Note that from the compositional data, the calculated particle density of an apple is about 1064 kg m^{-3}. Most apples float in water, indicating a density less than 1000 kg m^{-3}. Mohsenin (1986) quotes a value of 846 kg m^{-3}, suggesting an air content of about 20%. One important objective of blanching is to remove as much air as possible from fruit and vegetables prior to heat-treatment in sealed containers, to prevent excessive pressure development during their thermal processing. Data on the amount of air in fruits and vegetables are scarce in the food literature. There is evidence that this air is quickly displaced by water during soaking.

Data on particle densities are provided by Lewis (1990), Mohsenin (1986), and Hayes (1987). Note that if the food is frozen, the density of ice should be substituted (916 kg m^{-3} at 0°C). However, not all the water is likely to be frozen, even at –30°C.

The particle density of dehydrated powders is considerably affected by the conditions of spray drying. Increasing the solids content of the feed to the drier will result in higher particle densities and bulk densities. High particle densities will enhance sinkability and reconstitution properties. Differences in particle densities are exploited for several cleaning and separation techniques, e.g. flotation, sedimentation and air classification.

9.2.4 Forces of adhesion

There will be interactions between particles, known as forces of adhesion and also between particles and the walls of containing vessels. These forces of attraction will influence how the material packs and how it will flow. Some of the mechanisms for adhesive forces have been described as

- liquid bridging by surface moisture or melted fat;
- electrostatic charges;
- molecular forces, such as Van der Waals and electrostatic forces;
- crystalline surface energy.

Schubert (1987a) describes some of the models that have been used to quantify these forces, and the limitations of such models.

There is some indication that interparticle adhesion increases with time, as the material consolidates. Flowability may be time-dependent and decrease with time.

9.2.5 Bulk properties

Although the discussion so far has focused on individual particles, the behaviour of the collective mass of particles or bulk is very important in most operations. The bulk properties of fine powders are dependent upon geometry, size, surface characteristics, chemical composition, moisture content and processing history. Therefore it is difficult to put precise values on them and any cited values should be regarded as applying only to that specific circumstance, Peleg (1983).

The term cohesive is used to describe the behaviour of powders, as they are influenced by forces of attraction (or repulsion) between particles. For powders that are cohesive, the ratio of the interparticle forces to the particles' own weight is large. This ratio is also inversely proportional to the square of the particle size, which explains why small particles adhere to each other more strongly than large particles. Schubert (1987a) states that the majority of food particles are non-cohesive (and thus free flowing) only when the particle size exceeds 100 μm. Increase in moisture content makes powders more cohesive and increases the size at which the transition from cohesive to non-cohesive takes place.

Some of the bulk properties will be considered in more detail.

9.2.6 Bulk density and porosity

The bulk density is an important property, especially for storage and transportation, rather than separation processes. It is defined as the mass divided by the total volume occupied by the material. This total volume includes air trapped between the particles. The volume fraction trapped between the particles is known as the porosity (ε), where

$$\varepsilon = ps - pb/ps \tag{9.5}$$

where ps and pb are measured solid and bulk densities. Methods for determining bulk density are described by the Society of Dairy Technology (1980) and Niro (1978). Terms used depend upon the method of determination and include loose bulk density and compacted and compressed bulk densities.

Some bulk densities of powders are given in Table 9.7. Further values are given by Peleg (1983), Hayes (1987) and Schubert (1987a). Peleg (1983) argues that the relatively

Table 9.7. Bulk density of some powders

Powder	Bulk density (kg m^{-3})	Powder	Bulk density (kg m^{-3})
Oats	513	Milk	610
Wheat	785	Salt (granulated)	960
Flour	449	Sugar (granulated)	800
Cocoa	480	Sugar (powdered)	480
Coffee (instant)	330	Wheat flour	480
Coffee (ground and roasted)	330	Yeast (baker's)	520
Corn starch	560	Egg (whole)	340

From the data of Milson and Kirk (1980).
From the data of Peleg (1983).
Taken from Lewis (1990) (with courtesy of Prentice Hall).

low bulk density of many food powders cannot be explained solely by geometrical considerations. As mentioned, most food powders are known to be *cohesive*. Therefore open bed structures supported by interparticle forces are very likely to occur. Such materials are likely to have a low bulk density and high porosity. Factors that increase cohesiveness and interparticle forces are likely to decrease the bulk density. Moisture sorption tends to increased cohesiveness, mainly due to interparticle liquid bridges. Anticaking agents are believed to work by reducing cohesive forces and thereby increasing bulk density. Peleg (1983) provides data for the cohesiveness of some powders together with the effects of some anticaking agents on the bulk density of some food powders.

Powders can be compressed either by tapping or mechanical compression, as in tableting. The forces involved in compression are much higher than those in tapping or mechanical vibration.

The ratio of tapped bulk density to the loose bulk density is referred to as the *Hausner ratio*. Hayes (1987) quotes the following ranges, together with some values for some food powders:

1.0–1.1	free flowing
1.1–1.25	medium flowing
1.25–1.4	difficult
>1.4	very difficult.

Hayes also refers to another index, termed the 'Novadel Tap Test', which is related to the percentage volume decrease on tapping. The larger the volume decrease, the poorer is the flowability.

Peleg (1983) states that the Hausner ratio may be used for flowability index in powders, where friction is the major obstacle to flow, but that there is no evidence that it is useful for cohesive powders.

When powders are compressed the powder bed deforms and a number of mechanisms are involved, including spatial rearrangement of the particles without deformation

together with those brought about by fragmentation and plastic deformation of the particles. For cohesive powders, the open structure supported by interparticle forces is relatively easily overcome by the compressive force and there is a relatively large change of bulk density with pressure. Non-cohesive powders show relatively little change of bulk density with pressure.

An empirical relationship of the following form is found to fit experimental data well:

$$\rho_B = a + b \log s \tag{9.6}$$

where ρ_B is the bulk density, a and b are constants and s is the shear stress. The constant b is defined as the compressibility. High values of b indicate a cohesive powder, whereas low values indicate a non-cohesive powder. Some values for different powders are given by Peleg (1983). The use of anticaking agents was found to reduce the compressibility.

9.2.7 Flowability

The flowability of powders is very important in their handling. Some indices of flowability have already been discussed.

Generally flowability increases with increasing particle size and decreasing moisture content. As well as compressibility and cohesiveness, other factors used to assess flowability are as follows:

- *Slide angle.* This is measured by placing the powder sample on a flat smooth horizontal surface, which is then slowly inclined until the powder begins to move. The angle at which movement occurs is known as the slide angle.
- *Angle of repose.* This is useful in the design of powder handling systems. Its value depends upon the method of determination, which is usually by forming a heap. Other methods involve a bed rupture or a rotating drum method. Its magnitude is affected by frictional forces and interparticle attractive forces, which become dominant in cohesive powders.

  According to Carr (1976), angles of up to 35° indicate free flowability; 35–45° indicates some cohesiveness; 45–55° indicates cohesiveness or loss of free flowability; >55° indicates very high cohesiveness, very limited or zero flow

These parameters are empirical in nature and often the results are not applicable, when conditions are changed (Peleg, 1977).

Peleg (1977) and Schubert (1987a) have described a more fundamental method for looking at the flow behaviour of powders, based on the work of Jenike, described by Leniger and Beverloo (1975). A flow cell is used, where the powder is first consolidated to a particular bulk density and porosity (see Fig. 9.3(a)). It is then subjected to a compressive force (N) and the shear force (S) required to cause the powder to yield and shear is determined. These readings are converted to a normal stress (σ) (N/A) and a shear stress (τ) (S/A). This procedure of determining the shear stress is repeated for a number of different normal stress values. The information is presented on a plot of shear stress against normal stress and gives the yield locus, for that particular porosity.

Figure 9.3(b) shows the data obtained for a non-cohesive powder, which can be characterised by the angle of friction (α). Also in all cases a large angle of friction,

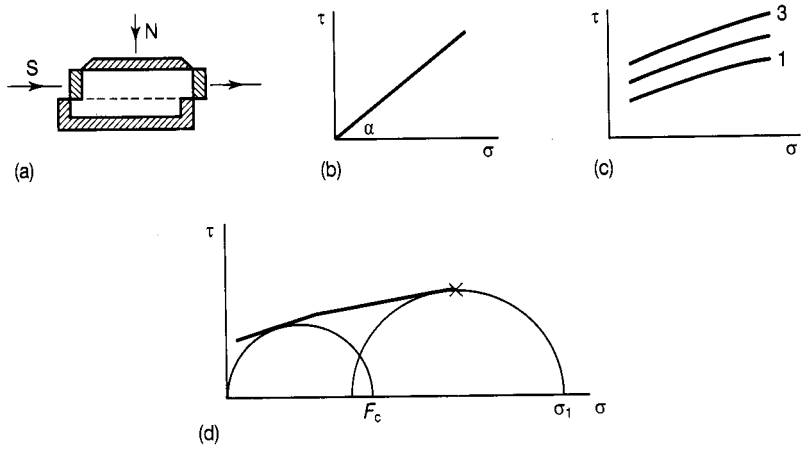


Fig. 9.3. Solid characterisation: (a) Jenike flow cell; (b) normal stress against shear stress, for a non-cohesive powder, α = angle of friction; (c) yield locus for a cohesive powder for powders compacted to different initial porosities; porosity $1 > 3$; (d) Mohrs circles, showing the unconfined yield stress (f_c) and major consolidation stress (σ_1).

indicating high interparticle friction, does not always mean poor flowability, for example dry sand has a high value but flows quite well.

Figure 9.3(c) shows the yield locus for a cohesive powder, at a particular porosity. However, if the porosity of the sample is increased, the yield locus will change. Therefore there are a family of curves at different porosities. Also the curves do not pass through the origin. This yield locus data therefore describes the flow behaviour of powders.

This data is used to determine the unconfined yield stress (f_c) and the major consolidation stress (σ_1), by application of Mohrs circles (see Peleg, 1977; Schubert, 1987a; Leniger and Beverloo, 1975).

The ratio of σ_1/f_c is termed the Jenike flow function, which has also been used as an indicator of the flowability of powders. Its values correspond to the following characteristics:

<2	very cohesive, non-flowing
2–4	cohesive
4–10	easy flowing
>10	free flowing.

This more fundamental information is extremely useful for designing hoppers, bins, pneumatic conveying systems and dispensers. Similar measurements can be made using a more sophisticated annular flow cell, which is capable of reliable shear force determinations at low normal stresses.

The hydrodynamics of powder flow are different to that for liquids. The pressure does not increase linearly with height, rather it is almost independent. Also they can resist appreciable shear stress and can, when compacted, form mechanically stable structures

that may halt flow. Also any pressure or compaction can increase the mechanical strength and hence the flowability.

9.3 SEPARATION OF PARTICULATES AND POWDERS

This section will be most concerned with the separation or recovery of solids from within a solid matrix or from a particulate system. The main emphasis will be those in fine particulate form, so the production of material in a form suitable for separations is often crucial for the process. In this respect size reduction and milling equipment is important.

9.3.1 Size reduction

Size reduction is a very important preliminary operation for separation processes for many cereals, legumes and other commodity crops, as well as for extraction operations, e.g. tea and coffee, or expression processes, e.g. fruit juice expulsion or oil extraction.

Sugar is one example of a commodity that comes in a range of particle sizes, e.g. granular, caster, and icing sugars. Some data on sieve size measurements of different sugars is cited by Hayes (1987).

The term 'crushing' is applied to the reduction of coarse material down to a size of about 3 mm, whereas 'grinding' is commonly used for the production of finer powdered material.

The degree of size reduction can be characterised by the size reduction ratio (SRR), where

$$\text{SRR} = \frac{\text{average size of feed}}{\text{average size of product}} \tag{9.7}$$

Several stages may be required if the overall size reduction is large.

The main forces involved are compressive forces, impact forces and shear or attrition forces. Usually there is a predominant force involved for each type of equipment, although the other forces may be involved to a lesser extent. The fracture resistance increases with decreasing particle size.

Aspects which need to be considered in the selection of the most appropriate equipment for size reduction are the particle size range required and the hardness of the material. Hardness can be measured in Mohs, whose scale ranges between 0 and 8.5. On this hardness scale, most foods are either very soft (<1.5 Moh); soft (1.5 to 2.5 Moh) or medium hard (2.5 to 4.5 Moh). More details are provided by Hayes (1987) and Christison (1991).

Very soft materials such as dried fruit, dried plant material, meat and fish may be processed with a Colworth stomacher down to 100 μm, or high-speed cutters, such as a bowl choppers.

Other mills for processing grain cereals, legumes, salt, and sugar include the following:

(1) *Hammer mills.* These are very much general-purpose mills. Size reduction is mainly due to impact forces. They are widely used for peppers and other spices, sugar and dried milk powder.

(2) *Roller mills.* These can be one or several sets of rollers; size reduction is by

compressive forces; size reduction ratio is usually below 5. These are widely used for the milling of wheat and refining of chocolate. (Size range 10–1000 μm.)

(3) *Disc attrition mills.* These come in a number of designs. Simple disc mills have two discs, one of which is stationary and the other moving. The speed is relatively slow, with a peripheral velocity of 4–8 m s^{-1}. The feed material enters at the centre of the discs and the discs are profiled in such a way as to cause grinding to occur as the material falls radially across the grinding discs. (Size range down to 100 μm.)

On the other hand, impact pulveriser mills, such as pin disc or stud mills, operate at high rotational speeds, creating peripheral velocities up to 200 m s^{-1}. In this case the discs contain pins or studs, which intermesh. In the simple design there is one stationary and one moving disc, whereas in other designs both discs move.

These types of mill can produce very fine powders, suitable for air classification (see Section 9.4).

Another high-speed mill is the high-speed rotor mill, which is a variant of the hammer mill. A rotor with a series of hardened blades rotates at speeds in excess of 15 000 r.p.m. and the fines pass through a sieve ring, fitted round the circumference.

(4) *Ball mills.* This is a tumbling mill and is used for very fine grinding processes. It comprises a horizontal slow-speed rotating cylinder which contains steel balls of flint stones; the balls are normally 25–150 mm in diameter. The mechanism is by impact and shear. The optimum speed of rotation is about 75% of the critical speed, which is defined as the speed which causes the steel balls to centrifuge.

Two or more mill types may be required to achieve the desired level of size reduction.

The size reduction achieved often depends on whether the discharge product is released immediately or whether it is restricted by use of a screen. In the latter case the residence time within the action zone is increased until the particle is smaller than that of the screen. A third alternative is to allow all the particles to leave unrestricted and to separate them externally, recycling oversize particles for further milling.

The particle size required also affects the cost of milling and the energy requirement: the latter is based on the following equation:

$$\frac{dE}{dD} = \frac{K_m}{D^n}$$

where dE is the energy required to produce a small change in diameter dD and K_m is a characteristic of the material. The three main equations result from different values of n. (*Note*: n is a power-law exponent.)

$$n = 1 : E = K_m \ln\left[D_1/D_2\right]; \qquad \text{Kick's law}$$

$$n = \tfrac{3}{2} : E = 2K_m\left[\frac{1}{D_2^{0.5}} - \frac{1}{D_1^{0.5}}\right] \qquad \text{Bond's law}$$

$$n = 2 : E = K_m\left[\frac{1}{D_2} - \frac{1}{D_1}\right] \qquad \text{Rittinger's law}$$

where D_2 is the final diameter and D_1 is the initial diameter. Energy requirements are well in excess of those required to produce a new surface, as much energy is lost in friction and other inefficiencies. Care must be taken to ensure that the sample does not get too hot during size reduction.

Wet milling can be achieved by wetting the material and the feedstock is ground in a suspension in the liquid, which is often water. Energy requirements are usually slightly higher than for dry milling but a finer powder is obtained and dust problems are eliminated. The wear and tear on the mill is also higher. Often wet milling is useful as part of an extraction process, whereby soluble components are transferred from the solid to the liquid phase. Wet milling is popular for corn milling.

More information on size reduction is provided by Brennan *et al.* (1990), Loncin and Merson (1979), and Christison (1991). One very pertinent comment about milling is that the weakness of a material may be at the juncture of different components, thereby initiating a crude form of fractionation process, which can be further exploited.

9.3.2 Sieving

Sieving is probably the easiest and most popular method for size analysis and separation of the components within powders. A sieve is an open container which has uniform square openings in the base.

The screen aperture is defined as the space between the individual wires of a wire mesh screen, and the mesh number is the number of wires per linear inch. However, this nomenclature has now been deleted from the latest British Standards although it may still be regularly encountered.

A whole range of standard sieves are available up to 25 mm in size. Sieves for powders and agglomerates may be from a few millimetres, down to about 20 μm. Sieves in common use are produced to a number of standards, e.g. BS 410,1969 (see Table 9.8), ISO R 565 and ASTM E11 81. In most cases the screen interval, which is the ratio of successive sizes in a test series, is either 2, $2^{0.5}$ or $2^{0.25}$.

The high mesh sieves may be too fragile for some applications. A more robust set consists of the Institute of Mining (IMM) screens, where the thickness of the wire is approximately the same as the aperture size. The Tyler series is another used in America. Coulson and Richardson (1978) summarise these. Hayes (1987) also gives a summary of the most widely used mesh sizes, the smallest being about 44 μm. These sizes are slightly higher than both the British and American standards. Some information is also provided by Christison (1991). A number of special sieves are available for particle size measurement below 50 μm. Complications arise below this size for reasons discussed later.

A single sieve separates a particulate material into two fractions. When sieving materials of a non-spherical nature, the situation is complicated by the fact that particles with a size close to that of the nominal aperture of the test sieve may pass through only when presented in a favourable position. Such particles, sometimes termed 'near-aperture particles', may partially block or blind the sieve aperture and reduce its effective area. Therefore some particles less than the nominal sieve diameter will be retained by the sieve. Particles much smaller than the sieve nominal diameter pass through fairly rapidly, whereas those close to the sieve diameter take a much longer time and a small fraction may never pass through. The effectiveness of a sieving process depends upon the amount

Table 9.8. Common mesh sizes

Mesh size	Aperture (mm)	Mesh size	Aperture (μm)
4	4.00	44	355
6	2.80	52	315
7	2.24	60	250
8	2.00	72	224
10	1.80	85	180
12	1.40	100	160
14	1.25	120	125
16	1.00	150	112
18	0.90	170	90
22	0.71	200	71
25	0.63	240	63
30	0.50	300	56
36	0.45	350	45
		400	36

of material placed on the sieve, the type of movement imparted to the sieve and the time allowed for the process. A small charge will result in more effective sieving, but care should be taken to ensure that a uniform sample is taken, particularly for analysis. The criteria may well be different for analysis and separation. The sieving time can be affected by the following factors (British Standards Institute 1796, 1989):

- the material characteristics, e.g. fineness, particle shape, size distribution, density;
- intensity of sieving;
- nominal aperture size of the test sieve;
- characteristics of sieving medium;
- humidity of the air.

Materials can also be sieved in a liquid, the procedure being referred to as wet sieving. Wet sieving is used for extremely fine particles, e.g. below 50 μm, or particles that become electrically charged. One advantage is that it reduces inter-particle adhesion. Also powders that cannot be dispersed or materials in liquid suspension should be sieved wet in order to facilitate dispersion of the primary particles, which may cause the coarser particles to agglomerate or may be difficult to disperse. A wide variety of dispersant liquids is available, for example ethanol, isobutanol or benzene, for wheat flours, and octanol for milk powder. More details are provided by Schubert (1987a).

Usually a number of sieves are clamped together, with the largest on the top. The material is placed in the top sieve and the sieves are subjected to a vibratory mechanism. In this way a distribution of particle sizes can be determined. This method is recommended for particle size estimation between 100 and 1000 μm. More sophisticated equipment available for research and development relies on vibrations caused by electromagnetic or sonic mechanisms (Christison, 1991).

A special type of sieve is the *air jet sieve*, in which a reduced pressure is applied to the underside of the sieve. A jet of air is discharged upwards from a radial slotted arm rotating continuously under the screen and this helps prevent blinding by fluidising the particles that are likely to cause blinding. A diagram of the air jet sieve is shown in Fig. 9.4. One separation is performed at a time. This is very useful for analyses involving smaller particles.

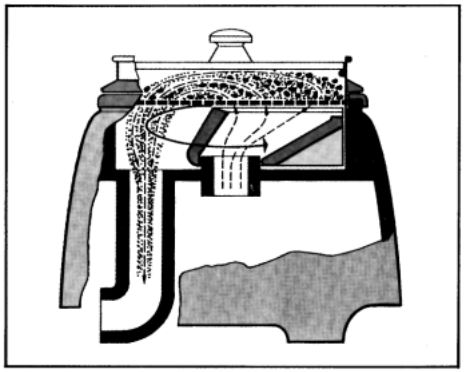


Fig. 9.4. Air-jet sieve (courtesy of Hosokawa Micron Ltd).

Test sieving is carried out on a wide variety of food materials and for many different purposes, for particle size analysis, for producing materials with a known particle size distribution or for supplying materials of a specified size range. Problems encountered with sieving result from sample stickiness, sieve blockage and agglomeration. Strumpf (1986) contests that these problems increase exponentially as the sieve size decreases.

One of the main applications of sieving is in the flour industry, to separate the different fractions of flour. Names of particular sieving processes include scalping, to separate the break stock from the remainder of the break grind; dusting, bolting and dressing, which involves sieving flour from the coarser particles; and grading, which is classifying mixtures of semolina, middlings and dunst into fractions of restricted particle size range. Semolina, middlings and dunst are terms used to describe fractions of rolled endosperm of decreasing particle size (as determined by sieving). Definitions are provided by Kent (1983).

Other terms sometimes used are scalping, for removing large particles, and dedusting, for removing small particles, Brennan *et al.* (1990).

9.4 AIR CLASSIFICATION

9.4.1 Introduction

Air classification is a means of using a gaseous entraining medium, which is usually air, to separate a particulate feed material into a coarse and fine stream, on a dry basis. According to Klumpar *et al.* (1986), classifier designers take advantage of the following phenomena to achieve this objective:

- small particles fall more slowly in air than large particles;
- larger particles have a greater centrifugal force in cyclonic flow than smaller particles;
- smaller particles have less inertia and can change their direction of flow easier than large particles;
- larger particles require a higher conveying velocity;
- larger particles have a larger probability of colliding with a rotating blade.

Separation is based mainly upon particle size, although other particle properties, such as shape, density, electric, magnetic and surface properties may play a part. The procedure of winnowing or aspiration is a traditional process to separate chaff from grain after threshing and is one of the simplest forms of air classification. The chaff is dispersed in the wind or by using an air stream. This principle is used on vining and combining machines for harvesting peas and grain. Another simple form of classification involves subjecting a powder, containing a range of particle sizes, to an upward airstream of constant and uniform velocity (Fig. 9.5(a)). Some of the particles will become fluidised, some will be conveyed and carried away by the air stream and others will remain stationary. Consequently, some degree of separation is achieved. In principle, the remaining material could then be subjected to a higher velocity, removing another fraction, and this process could be continued. The forces involved in this simple process are the drag forces acting on the particles due to the air stream, which counteract that due to gravity.

An alternative system is to use a long shallow, slightly sloping tray (see Fig. 9.5(b)). This type of separation also forms the basis of the simple zig-zag separator, illustrated in Fig. 9.5(c) and Fig. 9.6, which can be single or multiple tube. This is used for separating particles in the range 0.1 to 10 mm. In this case the separation is further enhanced by tortuous passages and collision surfaces, which are particularly effective at removing the larger and more dense particles. It can be used for dedusting operations and is also capable of removing shells or hulls from disintegrated peanuts, cottonseed, rapeseed or cocoa beans.

However, the major interest in air classification is that it provides a means of separating small particles which cannot be readily achieved by sieving, i.e. below 50 μm. Thus a

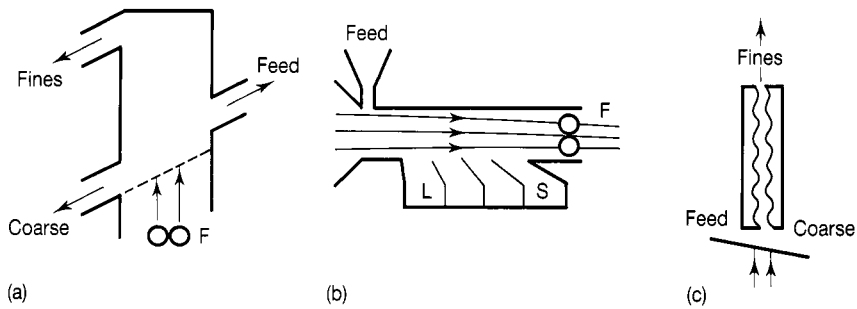


Fig. 9.5. Simple classifiers: (a) for aspiration F = fan; (b) for fractionation L = large; S = small particles; (c) zig-zag classifier.

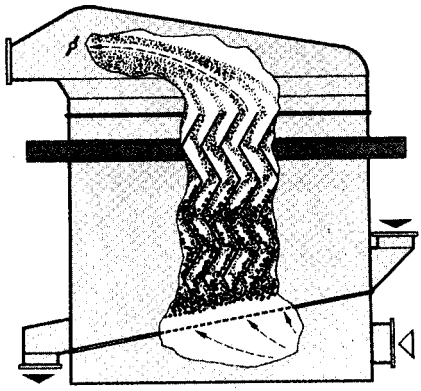


Fig. 9.6. Commercial zig-zag classifier (courtesy of Hosokawa Micron Ltd).

powder subject to air classification can be separated into two streams, one primarily below the required particle size and the other predominantly above it. The required particle size is referred to as the cut size and is discussed in more detail in Section 9.4.3. Cut sizes of interest in food processing operations may range between 2 and 50 μm.

9.4.2 Commercial air classifiers

In many commercial air classifiers, the gravitational force used in the examples cited above is supplemented by a centrifugal force, induced by subjecting the particles to circular motion. This is essential for separating small particles and speeds up the separation process. This centrifugal force is produced by a spiral or vortex air flow pattern, promoted by a tangential air inlet and the use of directional vanes or baffles. In addition there may also be a rotating disc or turbine, which further accelerates both the air and particles and increases the centrifugal force produced. This force acts toward the outside of the chamber, and counteracts the drag force which is produced by the air moving in a spiral direction toward the centre of the classifying chamber.

The mode of operation of a typical classifier is as follows. The inlet air is mixed with the material to be separated. The feed particles are subjected to a centrifugal force originating from a revolving rotor and a drag force produced by the air current, which moves in a spiral direction toward the central shaft. The separation is based on differential mass, density and shape. The larger and more dense particles are influenced by the mass-dependent centrifugal forces and move toward the outside of the chamber, where they are removed by a discharge worm-screw conveyor or some other means. The smaller, lighter particles are more subject to the frictional forces of the air current and move with the air stream, leaving from the centre of the classifying chamber, into a cyclone, where they are separated from the air. The relative magnitude of the two forces can be changed by altering the rotational speed of the disc and the air velocity; changing either will change the cut-size. Classifiers with the facility to change these factors independently, will be capable of improved operational flexibility. Although the bulk of the separation takes place within the classifying chamber, some preliminary removal of the coarser particles may be achieved outside the main classifying chamber. The disc or

turbine can be mounted on a horizontal or vertical axis. The latter produces a centrifugal force in the horizontal plane and favours greater throughput, but less precision in cut-size (Fedoc, 1993). Some large capacity classifiers have several turbines mounted in one unit.

Air classifiers are categorised by reference to a number of factors, such as:

- the forces acting upon the particles; e.g. the presence or absence of a rotor, the drag force of the air and the presence of collision forces, which hinder larger particles;
- the relative velocity and direction of the air and particles, controlled by their respective feed systems;
- directional devices such as vanes, cones or zig-zag plates, which allow a change in direction and provide collision surfaces;
- location of the fan and fines collection device, whether they are situated internally or externally.

Other important features are the capacity of the classifier and the energy utilisation. A comprehensive treatment of classifier types and their operating principles has been reviewed by Klumpar *et al.* (1986). For some classifiers processing coal dust and cement, flow rates of over 100 tonnes h^{-1} can be handled. Laboratory classifiers are available which will handle batches of as little as 50 g of samples and which will separate 2–6 kg h^{-1} on a continuous basis. Larger classifiers handling foods can process more than 5 tonnes h^{-1}.

One machine which has been widely used for food processing is the Alpine Mikroplex classifier (Fig. 9.7), which is rated up to 1.6 t h^{-1} and a maximum energy consumption of 19 kW. This design uses a rotor on a horizontal axis. Material is fed by gravity between the fan and the vertical distributor plate. The air flow rate and direction of flow can be changed by vanes within the machine, and this is sufficient to change the cut size, which is also affected by feed rate. The cut size range is 3–30 μm. The coarse material is removed by a discharge worm conveyor. It is categorised as a free vortex machine. Such machines are now largely being replaced by forced vortex machines (Fedoc, 1993),

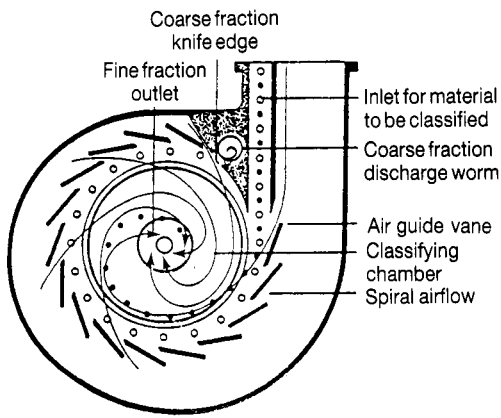


Fig. 9.7. Alpine Microplex classifier (frontal cross-section view) (courtesy of Hosokawa Micron Ltd).

whereby the force is provided by means of a rotor or turbine, which disperses the particles into an air stream applied by a suction fan. These types of equipment operate under a slight vacuum. Some examples are shown in Fig. 9.8.

9.4.3 Process characterisation

In most cases, air classification work is empirical in nature because of the difficulties in quantifying the forces acting upon a particle, with any degree of accuracy.

One method of characterising the separation is by means of the cut size. Ideally, all particles below the cut size end up in the fines and all particles above the cut size end up in the coarse stream (see Fig 9.9(a)). However, there will always be a small fraction of particles smaller than the cut size in the coarse stream and an equally small proportion of particles larger than the cut size in the fines stream. The extent of this overlap and the cut size can be determined by measuring the particle size distribution of the coarse and fine streams (see Section 9.2.2), and presenting the data for both streams as a weight frequency distribution. *The cut size is defined as that size where the weight of particles below the cut size in the coarse fraction is the same as the weight of coarse particles above that size in the fines stream* (see Fig. 9.9(b)). The yields of fines (Y_f) and coarse (Y_c) streams need to be known. If they are equal, the point of overlap (Fig. 9.9(b)) gives the cut size. If they are not equal, which is most likely to be the case, the frequency distribution for the fine stream must be multiplied by the yield for the fine stream, and that for the coarse stream by the yield for the coarse stream (Fig. 9.9(c)). The cut size is given by the point of intersection of these curves.

Factors which influence the cut size are the dimensions of the classifying chamber, peripheral forces and the spiral gradient. The cut point can be adjusted by varying the rotor speed, air velocity, vane setting and feeding rate.

By equating these forces when they are in equilibrium, an equation for the cut size (d) can be derived. This is based on Stokes' equation:

$$d^2 = \left[18\mu v_a r / \rho v_P^2\right] \tag{9.8}$$

where

μ = viscosity of air

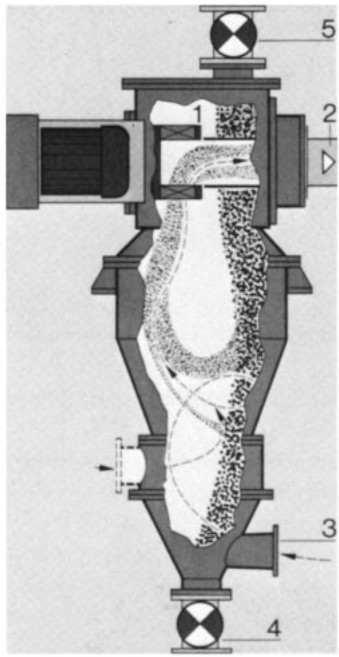
v_a = radial speed of air

r = clearance of classifier wheel

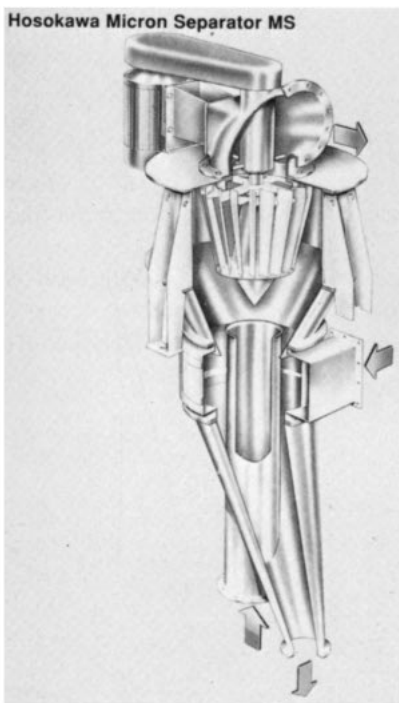
ρ = particle density

v_P = peripheral speed of particle (equivalent to rotational speed).

Although it is not possible to predict the movement of a particle by Stokes' law in an air classifier, because the forces acting upon the particle depend upon its position in the classifier, the equation is useful in that it predicts how the two main parameters, air flow rate and rotational speed, may influence the cut size. It predicts that increasing the air flow rate (v_a) increases the cut size, whereas increasing the rotational speed (v_P) decreases the cut size. The cut size for most operations is in the sub-sieve size range.



Turboplex Ultra-Fine Classifier



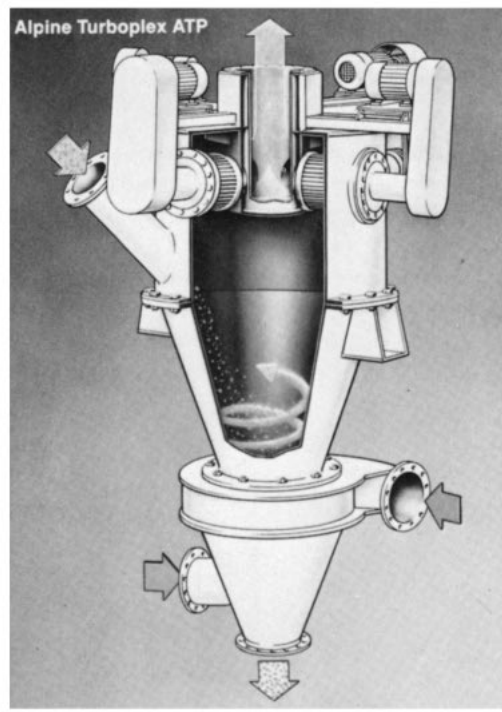


Fig. 9.8. Selection of air classifiers (courtesy of Hosokawa Micron Ltd).

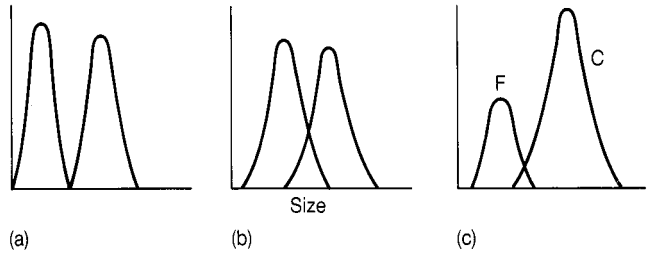


Fig. 9.9. Cut size determination: (a) ideal separation; (b) real separation, weight frequency distribution; (c) weight frequency distribution corrected for yield.

Therefore air classification provides an excellent means of separating powders, based on cut sizes below 40 μm.

However, the cut size alone does not provide information on how sharp the separation is. An alternative method of evaluation is to determine the grade efficiency, which also has the advantage of indicating the sharpness of the separation.

The particle frequency distribution is determined by weight for the coarse stream ($q_c(x)$) and feed material ($q_f(x)$) (see Fig. 9.9(b)).

The yield is determined for the coarse stream Y_c.

The grade efficiency $T(x)$ indicates for any particle size x, the mass fraction of feed material appearing in the coarse fraction. Thus

$$T(x) = \frac{Y_c q_c(x)}{q_f(x)}$$

Thus grade efficiency can be plotted against particle size (see Fig. 9.10).

The *cut size* is where the $T(x) = 0.5$, indicating the size of the particles, half of which appear in the coarse stream and therefore by difference, half of which appear in the fine stream.

The sharpness of the separation is measured by the ratio $k = [x_{25t}/x_{75t}]$, i.e. the ratio of the sizes giving grade efficiencies of 0.25 and 0.75 respectively. Ideally $k = 1.0$.

The best industrial air classifiers achieve $k = 0.7$, but typically commercial air classifiers show k values from 0.3 to 0.6 (Schubert, 1987b).

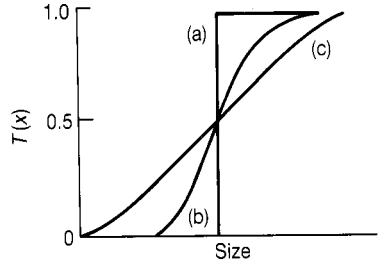


Fig. 9.10. Grade efficiency vs particle size: (a) ideal separation; (b) and (c) decreasing sharpness.

Another factor used to define the separation achieved is the *protein shift*, which is widely used in those operations where fractionation of protein occurs. The protein shift (S_p) for a simple separation is defined as

$$S_p = \frac{(C_p - C_{po})}{C_{po}} Y \tag{9.9}$$

where C_p = proteins in fines, C_{po} = protein in flour and Y is the dry weight yield of fines.

This can be determined by analysis of the resulting streams. Some values are shown in Table 9.9. Schubert (1987b) has shown that protein shift is largely independent of yield.

Table 9.9. Protein shifts produced by air classification of flours from different cereals and legumes

Flour	Processing	Protein shift (%)
Barley	Roller-milled	19
Barley	Pin-milled	28
Malted barley	Commercial grind	8
Malted barley	Pin-milled	18
Oats	Pin-milled	27–32
Triticale	Pin-milled	28–36
Rice	Turbo-milled	8–10
Potato	Pin-milled	22–25
White bean	Turbo-milled	22
Field bean	Pin-milled	42
Fababean	Pin-milled	45

Taken from Sosulski (1983a) (with courtesy of Chapman Hall).

In an ideal separation all the protein will finish up in one stream, i.e. the fines. Under these conditions, the ideal protein content in the fines ($C_{p_{ideal}}$) will be C_{po}/Y, provided the yield is greater than the initial protein content.

It is possible to compare the measured separation with the ideal case by defining an efficiency of protein enrichment (epe), where

$$\text{epe} = \frac{(C_p - C_{po})}{(C_{p_{ideal}} - C_{po})}$$

Values range from 0 to 1, the higher the value the more efficient is the separation process.

Eliminating ($C_{p_{ideal}}$) gives

$$\text{epe} = \frac{(C_p - C_{po}) \cdot Y}{C_{po}(1 - Y)} \tag{9.10}$$

However, this is little used, compared to protein shift.

Schubert (1987b) gives an alternative method of analysing the data, using the following relationship:

$$C_p = \left[C_{po}/Y^m\right] \tag{9.11}$$

where m is a measure of the separation. If the protein content in the fines is plotted against the yield (Y), a characteristic curve is obtained, which can be used to determine m.

$m = 1$ applies to an ideal curve, where $C_p = \left[C_{po}/Y\right]$

$m = 0$ no separation, $C_p = C_{po}$

This approach can also be used to measure the effectiveness of the separation. Higher values of m imply better separation.

Examples are given for protein extraction from spent grain by air classification ($m = 0.12$) and wet processing ($m = 0.48$). The reason that wet processing is more effective is that the protein strongly adheres to the larger husks and is not effectively moved by air classification. The presence of a liquid breaks down these adhesive forces and improves the quality of the separation.

9.4.4 Applications

Air classifiers are designed to grade endosperm and cotyledon particles in the subsieve size range of 2–60 μm, into subgroups based on differential mass, density and shape. This is below the particle size conveniently handled by sieves. With most cereals the separation of starch and protein is based primarily on size and shape rather than density, even though the density difference is significant. However, these subgroups may still represent subcellular structures which differ substantially in their physical and chemical properties.

Most of the applications have been concerned with fractionation of the components of cereals and legumes, in particular the starch and protein fractions. The simplest process involves a single pass through the air classifier after size reduction.

An alternative procedure is to have a double pass, where the coarse stream from the first stage of separation is further milled and reclassified to produce a second fines stream (Fig. 9.11). This results in two fines streams, which may be handled separately or recombined, and one coarse stream.

Comparison of results from different workers is not straightforward, because not all the information is always presented. The quality of the separation will be influenced by the particle size range, cut point, yield, moisture content, feed rate, classifier type and operational conditions (see Section 9.4.3).

9.4.5 Cereal separations

Mature endosperms of most cereals are composed of thin-walled cells which are approximately 100 to 150 μm in diameter, with the cell walls being only 3–7 μm in diameter (Kent, 1983). Most of the cell volume is occupied by starch in the form of granules, which is embedded in a protein matrix. The main starch granules are spherical or lenticular in nature with a diameter 15–40 μm, although wheat, barley and oat endosperms also

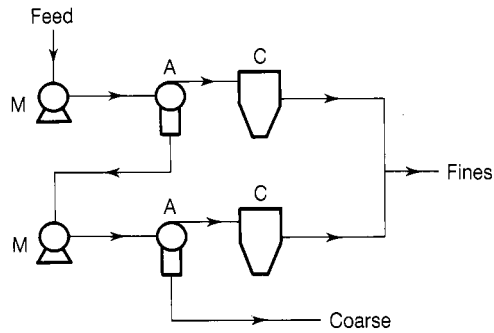


Fig. 9.11. Double classification process: M – mill; A – air classifier; C – cyclone.

contain a low proportion by weight of small spherical granules with a diameter of 1–10 μm. The protein matrix is often referred to as interstitial or wedge protein. In cereal flour milling it was discovered at an early stage that the finer flour contained more protein, which led to the development in some countries of commercial procedures for protein displacement milling of soft wheat flours, whereby finely ground flours were air classified into a light fine fraction which has double the protein content of the original wheat flour and a coarse starch fraction with some specific advantages in certain applications. Obviously the quality of the separation depends upon the cereal type and variety and the method of milling.

During conventional roller milling of wheat, the endosperm particles are separated from the bran while progressively being reduced in size to pass through sieves having apertures of 100–150 μm. The resulting flour consists of particles with a range in diameter of 2–200 μm. Kent (1983) made some observations on the differences between hard wheat and soft wheat. Hard wheats contain higher protein contents 12–14% and are physically more difficult to break. Soft wheats have a lower protein content and are more easily reduced in size. In hard wheats, the majority of the particles are shattered cell fragments over 50 μm in diameter, whereas in soft wheats the protein matrix is partially disintegrated to yield more of the detached starch granules and wedges of protein matrix containing variable amounts of small starch granules and cell wall fragments. There was also a difference in the amount of protein bound to starch between hard and soft wheat.

It is now common practice to use impact mills such as the pin disc mill on powders as a pretreatment to air classification, as these are capable of producing significant proportions below 20 μm. Impact mills consist of two sets of pins. In the simple design only one set of pins rotates. Finer flours result when both sets rotate in opposite directions at different speeds (6000–18 000 r.p.m.). An optimum impact velocity for disintegrating most endosperm is about 200 m s^{-1}, which is not high enough to damage the starch granules extensively. Damaged starch granules would adversely affect the baking performance and functionality of the starch fraction. Impact mills are best for soft and brittle products because of the high wear and tear. Particle size was also found to be dependent on moisture content, feed rate, nature of the grinding surface and size of the screen selected.

Jones *et al.* (1959) showed that there were three principal groups of particles in pin-milled wheat flour: large particles of either cell wall material including starch and protein, or larger detached starch granules or seed coat (>40 μm); medium-sized starch granules, some with adherent protein (15–40 μm), and small chips of free wedge protein and detached small starch granules (<15 μm). The main mechanism of separation was size rather than density differences.

Kent (1965) fractionated hard (13.6% protein) and soft (7.6% protein) wheats, using 17 and 35 μm as the cut-off to distinguish between the fractions. The yield of the fine fraction (0–17 μm) was 7% for the soft wheat, but only 1% for the hard wheat and the protein content in the fine fraction had increased much more for the soft wheat, to 14.5%, compared to 17.1% in the hard wheat. The yield of intermediate fraction for soft wheat (17–35 μm) was 45% and the protein fraction was reduced from 7.6 to 5.3%. The yield of the coarse fraction (over 35 μm) was 48%, but the protein content was higher than the original, at 8.9%. Further size reduction, using an impact mill, increased the fine fraction yield for the soft wheats from 7 to 20% and the protein content from 14.5 to 15.7%. Thus the amount of protein associated with the fine fraction had increased by 300%. For wheat it can be seen that a limited fractionation of its major components can be achieved and that the protein content can be doubled in the fine fraction for soft wheats.

As cut size increased above 19 μm, the yield of fines increased but the protein content of the fines started to decrease (Jones, 1960; Sullivan *et al.*, 1960). Repeated classification, performed by remilling the coarse fraction and reclassifying it at the same cut point (four times) produced protein shifts ranging between 20 and 34%, and (eight times) ranging between 30 and 60%. These improvements were more marked for soft wheat than for hard wheat (Stringfellow *et al.*, 1963–64).

One problem could be starch damage. Some of the protein was found to be bound to the starch. Sosulski (1983) summarises the use of air classification for cereals. Since then, more attention has been devoted to hard wheats. For these, attrition milling has been found to be more effective than pin disc milling, and protein enriched fractions have been used as gluten replacers (Sosulski *et al.*, 1988a). Further work on hard wheat classification and baking properties of the resulting fractions has been reported by Nowakowski *et al.* (1987).

Vose (1978) provides data on some protein shifts for barley, malted barley and oats (see Table 9.9). Protein shifts were higher for flours produced by pin-milling.

9.4.6 Legumes

Sosulski (1983a) has reviewed the use of air classification for fractionating proteins in some legumes. He produced data which gave a measure of the efficiency of the process, in comparison with those for some cereals. Comparison was made using the degree of protein shift, which is based on the yields of coarse/fine fractions and their protein contents relative to the parent flour (see eq. (9.9)). Some values are given in Table 9.9.

High protein shifts are indicative of good separations. The results confirm that pin-milling as a pretreatment improves the quality of the separation and roller-milling and turbo-milling are less effective because they fail to release much protein. Results with air classification of potato protein were not very promising, but the protein shifts of greater than 40% for legumes were very encouraging. Unlike most cereals, the starch granules of

legumes do not exhibit a bimodal distribution or variable distribution and the average dimensions are 16–21 μm in width and 23–28 μm in length (Biliaderis *et al.*, 1981). Pin-milling has no effect on the starch granules but reduces other cell structures to below 5 μm.

Trials with field peas, which contain 14–27% protein, have proved successful and Sosulski (1983a) reported results for separation of field peas containing 21% protein, into 25% of fines, containing 60% protein. The coarse fraction was pin-milled a second time to release more protein and reclassified, giving an additional 10% of fines, containing 46% protein. The resulting final coarse fraction contained only 3% protein, giving an overall recovery of over 90%. Further pin-milling and reclassification, in order to further reduce the protein content of the starch fraction and improve its functionality, resulted in marginal reduction in the protein, but considerable increase in the amount of starch damage. The residual protein could also be removed by water washing, which gave a product containing less than 0.06% nitrogen.

Experience has shown that it is not necessary to remove the hulls prior to pin-milling. However, if not removed, they will finish up in the coarse fraction and modify its composition, as they comprise a significant part of the seed weight, about 8% for field peas and 13% for fababeans. They may cause problems if they contain anti-nutritional factors and it is advisable to remove them for fababeans to avoid problems from condensed tannins.

Fababeans contain 28–32% protein, which is substantially more than field peas and makes them an attractive proposition for air classification. They have also been successfully fractionated.

Work with field beans showed that protein fractions at yields of 20–30% and containing 50–60% protein could be obtained in the fines. However, the coarse fractions still contained substantial quantities of protein, giving a considerable reduction in the overall recovery. Results for a number of other legumes are recorded in Table 9.10.

Tyler *et al.* (1984) examined the effect of cut size on the separation of several legumes. In general an increase in the cut size resulted in an increased yield of the fines fraction and protein recovery in the fines, but a decrease in protein concentration. However, the amount of starch in the fine stream increased, together with a decrease in starch separation efficiency.

In fact, for legumes, air classification can be regarded as a means of separating starch, as most of the non-starchy materials finish in the fines (Han and Khan, 1990a). For pea flour it was found that most of the lipid fraction was found in the fine stream, but fibre was distributed between the fractions (Wright *et al.*, 1984). Cloutt *et al.* (1986) investigated starch size distribution for cowpeas, fababeans and pigeon pea and found considerable differences between them.

Cloutt *et al.* (1987) looked at the effects of cut size on the separation characteristics of the same three legumes. For each one there was a good relationship between the fines yield and protein content of the fines. This relationship could be established to permit protein content to be estimated from the dry weight yield.

Han and Khan (1990b) have evaluated the effects of dry roasting on the fractionation process and functional properties of the separated fractions and found for the protein fraction that roasting reduced nitrogen solubility index and foaming properties.

Table 9.10. Yield, composition and separation efficiency of legume flours, protein fractions (*P*I, *P*II) and starch fractions (*S*II) produced by air classification of legume flours, percent dry basis

	Flour composition		Yield of product			Protein content			Starch content			Separation efficiency	
Legume	Protein	Starch	*P*I	*P*II	*S*II	*P*I	*P*II	*S*II	*P*I	*P*II	*S*II	Protein	Starch
Soybean	52.5	4.7	72	—	28	54.2	—	50.5	—	—	—	—	—
Lupin	41.4	3.5	82	—	18	43.3	—	30.0	—	—	—	—	—
Chickpea	20.4	53.2	13	7	80	46.9	45.1	13.1	6.3	5.3	65.1	48.5	97.6
Navy bean	26.5	44.9	20	15	63	58.6	50.7	9.0	1.0	2.6	71.0	82.0	97.8
Northern bean	22.6	47.2	29	13	58	50.7	38.8	4.8	0.7	3.0	80.6	87.3	98.9
Cowpea	23.6	52.0	25	12	63	51.6	45.8	8.0	3.2	9.2	80.7	79.0	95.0
Lima bean	22.8	48.8	24	15	61	49.6	43.4	7.1	0.0	0.4	79.6	81.0	100.0
Field pea	23.0	54.2	22	12	66	59.2	51.8	6.2	1.6	3.7	81.5	81.3	97.9
Lentil	26.3	53.6	22	15	63	64.6	54.3	5.4	0.2	5.8	85.6	88.7	96.1
Fababean	30.4	51.3	21	16	63	71.3	63.8	7.2	0.7	4.0	81.7	86.7	96.7
Mung bean	25.8	52.5	27	12	61	61.4	52.5	4.6	2.4	11.2	82.5	89.4	96.5

Reproduced from Sosulski (1983a) (with courtesy of Chapman Hall).

The functional properties of air-classified legumes have been reported to be very good. These have been evaluated for field peas and fababeans by Sosulski and McCurdy (1987) for the fine fraction. They reported good solubility properties and that water holding capacity and oil absorption increased with protein content. They also showed good emulsification, whippability and foam stability. Han and Khan (1990b) evaluated the functional properties for the fine and coarse fractions. In general, they were affected by the heat treatment, the ratio of protein to starch in the fractions and the presence of other components such as lipids. Aguilera *et al.* (1984) considered that there was potential for using both the protein-rich and starch-rich fractions in extruded products.

Sosulski *et al.* (1988b) contrasted the relative efficiencies of wet and dry processing methods; dry processing resulted in 75–85% recovery of protein for field beans and 93–98% recovery of starch. The protein concentrates from dry processing had higher whippability and foam stability, but lower water hydration and oil absorption capacity.

For cowpeas, protein fractions produced by dry processing generally produced superior products to those obtained from wet processing (Ningsanond and Ooraikul, 1989).

The binding of phytic acid to protein was found not to be affected by dehulling or air classification (Carnovale *et al.*, 1988). Tyler and Punchack (1984) found that milling and air classification was not influenced by the state of maturity, when peas were harvested at different dates. Sosulski *et al.* (1987) reported that dehulling and air classification improved the storage properties of cowpea products.

9.4.7 Other applications

Air classification and sieving was found useful in the preparation of oat-bran, which is rich in beta-glucan (Wood *et al.*, 1989). It has also been used to remove gossypol from cottonseed protein (Quang *et al.*, 1988).

Potato granules produced by spray drying from a wet milling process have been successfully classified. Granules containing 10% protein were converted to a fine stream containing 38% protein (Fedoc, 1993).

Air classification of spent brewers grain is described by Schubert (1987b). The recovery of protein was poor because of the strong forces of attraction between the protein and the husks.

For rapeseed extracts, a shift of 11.5–17.2% protein in the fines was found. However, phytic acid and glucosinolates were also concentrated. (King and Deitz (1987)). It was also found that steaming, crushing and air classification was an effective method for removing fibre.

Ground amaranth has also been air classified, for addition to breads (Sanchez-Marroquin *et al.*, 1985).

9.5 WET SEPARATION PROCESSES

As well as dry separation, wet separation techniques are available, most of which are dependent upon differential solubilities and precipitation methods. Of special interest is the recovery of protein from a solid matrix.

9.5.1 Protein recovery

Food composition tables simply give the protein content of foods, so we may be forgiven for assuming that protein is a single entity. In fact most foods contain many protein fractions; therefore there are many objectives in recovering them, the main ones being as follows:

(1) to recover all the protein from foods or their by-products to improve functional properties and reduce waste;
(2) to separate proteins from toxic components within the food;
(3) to recover specific biologically active proteins, such as enzymes, insulin and hormones;
(4) to fractionate proteins; for example albumins are soluble in water and globulins in salt solutions.

Deutscher (1990) provides a thorough review of protein purification methods.

In terms of selling price, there may be up to seven orders of magnitude difference between some of the proteins currently available.

The solubility of a protein in solution depends primarily upon the properties of its exposed surface groups, the type of solvent, its temperature, pH and polarity level, i.e. dielectric constant, and the type and concentration of dissolved ions. Water is the simplest extractant, and its pH is adjusted to be well away from the isoelectric point of the protein, which is in the region of pH 4 to 5 for many proteins. Dilute solutions of neutral salts are also used for salting-in, as these are thought to interact with surface charged groups, thereby improving solubility. Once in solution, it may then be required to aggregate and precipitate the protein. Methods available are:

- lowering the temperature to reduce protein solubility;
- adjustment of pH to the isoelectric point;
- addition of non-polar solvents to reduce the attraction of surface polar groups with water, to encourage hydrogen bonding between surface polar groups;
- unfolding (denaturation) and hydrophobic interactions;
- addition of large quantities of very polar solvents, which also causes unfolding, hydrogen bonding between surface polar groups and hydrophobic interactions;
- increasing the levels of salts (salting out), whose ions bind more readily to water and allow hydrogen bonding between exposed polar groups;
- raising the temperature to cause thermal denaturation to take place.

The principles are reviewed by Brocklebank (1987). Some specific examples will be taken from legume and cereal processing.

9.5.2 Soya processing

The terminology used for the different grades of proteins includes flours, grits, concentrates and isolates. Concentrates and isolates are protein enriched, where concentrate applies to products with greater than 70% protein on a dry weight basis (dwb), whereas isolates applies to greater than 90%.

Soyabeans contain about 40% protein and 20% fat (dry weight basis). The beans are cracked, dehulled and flaked prior to oil removal by solvent extraction; the defatted flakes are toasted to remove solvent and inactivate the antinutritional compounds. Undesirable changes may also take place, such as colour development (darkening) and protein denaturation. These are reduced by minimising retention times and operating at low temperatures. Desolventisation is by direct steam heating or by passing superheated solvent vapour through the flakes, which evaporates the bulk of the remaining solvent from the flakes. Protein flakes contain about 50% protein. Flakes are further milled to produce grits or flour, the only difference being the final particle size. Concentrates (70% protein) are produced by one of three methods, which involve the washing out of non-protein material:

- extraction with alcohol (60–80%);
- extraction with water acidified to pH 4.5 to minimise protein loss;
- water extraction with highly toasted flakes to minimise protein extraction.

In all cases the aim is to maximise the extraction of sugars and other soluble components and minimise the loss of protein.

Isolates are produced by aqueous extraction of proteins at elevated pH, followed by isoelectric precipitation using acid, producing a protein curd, which after washing contains over 90% protein. Greater than 90% protein is extracted at pH 8. The pH of minimum solubility is 4.2–4.6, where about 10% of the protein is soluble and not recovered. Functional properties such as solubility, whipping ability, emulsification capacity and gelation can be modified by various chemical, enzymatic and thermal treatments, before drying. The proteins in soyabeans, and many other legumes and cereals are characterised by a variety of means, for example their solubilities in different solvents (see Section 9.4.5), or according to their molecular weight, determined by ultracentrifugation or SDS electrophoresis (Pearson, 1983; Deutscher, 1990). Note that isolates can be produced by other methods, such as ultrafiltration and diafiltration (Chapter 4), or ion exchange (Chapter 6).

The principle of minimum solubility has also been applied in the procedure for the production of spun soya fibres, which involves solubilisation at low pH, followed by extrusion into an acid bath. The fibres produced are further stretched in a heated bath.

Texturisation of soyabeans can also be achieved by thermoplastic extrusion, by use of high temperatures and pressures. At the end of the extruder barrel the pressure is released and expansion occurs.

Similar principles of washing out or solubilisation and isoelectric precipitation can be applied to the production of concentrates and isolates from rapeseed, cottonseed, lupins and other protein sources (Hudson, 1983). Sosulski (1983b) noted that rapeseed meal gave lower extraction rates, a poorer recovery at low pH and a darker product, compared to soyameal, with only about a 50% overall yield of protein. Glucosinolates and some of the other toxic components can be removed by 80% ethanol. Some lupin seed varieties have the highest protein contents amongst the legumes (up to 45%), but are extremely variable in their composition. They can also be high in alkaloids, which are bitter and need removing. In some cases the fat content may be greater than 15% (Cerletti, 1983).

There has also been considerable work done on extracting protein from leafy materials (Humphries, 1982).

9.5.3 Wheat protein

An interesting example for cereals is the separation of the protein fractions in wheat. Traditionally, wheat proteins have been classified according to their extractability in various solvents. Schofield and Booth (1983) describe five fractions which can be produced by sequential fractionation:

- Albumins (1) and globulins (2) are extracted in dilute saline; on dialysis with water the albumins remain soluble and the globulins precipitate.
- Gliadins (3) are soluble in concentrated aqueous alcohol.
- Glutenins (4) are extracted in dilute aqueous acid or alkali.
- A residual fraction (5) is not extracted under any of these conditions.

All fractions are heterogeneous, with overlap between the samples. The main fractions are the glutenins and gliadins, which each constitute about 35% of the total protein. Both these fractions are insoluble in water and a crude preparation can be produced by washing in water or saline.

The protein 'fraction' of greatest technological significance is *gluten*, produced by washing a flour dough with excess water. From the above discussion it would be expected to be heterogeneous in nature, comprising mainly glutenins, gliadins and residual protein, with small amounts of albumins and globulins. Although characterised as a protein fraction, it may also contain up to 20% of other components, such as starch, lipid and hemicelluloses, depending upon the production conditions. The gliadins confer extensibility while the glutenins and residue protein confer elasticity. More detailed analysis of these fractions is provided by Schofield and Booth (1983).

There is considerable demand for gluten products and production has expanded regularly as new uses for gluten and starch develop. Techniques involved in producing gluten are mostly based on extraction processes, from either a dough or a batter made from flour, rather than whole grain. In these processes, the gluten network is allowed to develop during the extraction process.

Requirements for raw materials are that the protein content should be high and the flour should be of consistent quality to ensure a uniform end-product. The other important raw material is water; with soft water the gluten is soft and slimy and the starch is removed less easily. Therefore hard water is preferred, perhaps suggesting a role for calcium in protein stability. Extraction from dough or batter uses considerable water and several alternative procedures have been investigated to reduce water utilisation.

Some processes rely on separating some of the starch before the gluten network fully develops. Centrifugation of flour slurry, not fully hydrated, produces (based on density differences) a starch-rich fraction and a second fraction containing most of the protein, with a protein content of 20–40%. This can be spray dried to produce a protein enriched fraction, but with a much lower protein content than normal gluten. Alternatively, the protein rich fraction can be further sheared and milled, which allows the gluten to develop, and this is removed by screens and further washed. Wet milling of whole wheat

has also been practised. Wheat grains are macerated with water, the bran screened-off and starch and gluten separated.

Other proposed methods involve chemical dispersion, for example in weak acids such as acetic acid, or in dilute ammonium hydroxide solutions. Non-aqueous separations have been proposed, using fluids with different densities, such as fluorinated hydrocarbons. Drying of gluten is important as gluten deteriorates rapidly if kept wet. Heat damage should be avoided to ensure good quality; quality is based on its protein content, physical characteristics and end-use performance.

9.5.4 Other applications

These examples illustrate some of the techniques that have been investigated to concentrate or recover protein from cereals and legumes. Similar approaches can be used for animal protein. Mackie (1983) gives a comprehensive review on the recovery of fish protein from a wide range of raw materials. Early fish protein concentrates were produced by solvent extraction to remove fat and water, followed by air drying to remove residual solvent. Bones may or may not be removed, leading to higher ash contents if they were not. One of their major problems was almost a complete lack of any functional properties. A similar procedure to that for soya, for the production of fish protein fibre from alkali extracts of fish processing wastes is also described. The handling of by-products from meat, fish and poultry are considered in more detail by Ockerman and Hansen (1988). Topics covered include mechanical deboning, rendering for fat extraction and waste meal production, and the extraction of gelatine and other food and non-food materials.

Another basis for separation is the ability of the water or solvent to reduce interparticle adhesion. Schubert (1987b) reports that interparticle adhesion is about one order of magnitude less in liquids than in gases. Therefore for strongly cohesive materials, there is potential for improving separations by dispersing the material in a liquid, followed by sieving or separating by sedimentation under those conditions. However, water costs and dewatering costs may be high. One example investigated was the extraction of protein from spent brewer's grain. This material contains about 20% dry matter, of which up to 28% is protein which has been heat denatured. This tends to adhere strongly to the much larger husks which contain little protein.

Wet processing using water to reduce interparticle adhesion was evaluated. Additional water was added and the mixture subjected to moderate shear and then separated. The protein enrichment was about 65%. Water utilisation was high. In an alternative process a specially designed screw press was used to separate the husks from the protein fraction.

The relationship between initial protein content, final protein content and yield could be described by the characteristic equation (see eq. (9.11))

$$C_p = \left[C_{po} / Y^m \right]$$

where $m = 0.48$. In both cases the degree of protein enrichment was much higher than for dry processing, whereby the whole mass was dried, milled and subject to air classification; the protein enrichment was between 30 and 40% and $m = 0.1$.

9.6 SOME MISCELLANEOUS SOLIDS SEPARATIONS

9.6.1 Dehulling

In many countries of the world, legumes are initially processed by removing the seed coat or hull and splitting the seed into its dicotyledenous components. Removal of the hull brings about some of the following advantages: a reduction in fibre and tannin content, and improvements in appearance, cooking quality, texture, palatability and digestibility.

Removing the hulls from many legumes is a tedious task. Legumes are often soaked and dehulled manually and redried. This method is probably the only one which removes all the hulls and consequently is used for estimating the hull content of seeds and also the theoretical yield of dehulled product, which usually range between 85 and 95%. Some values for hull content, expressed on a dry weight basis, are given in Table 9.11 (Reichert *et al.*, 1984).

Table 9.11. Dehulling performance of some different legumes

	Hull content % (a)	Yield (b)	Dehulling efficiency (DE) (c)	Hull adhesion (d)	Intact seeds % (e)
soya bean	8.27	88.7	0.72	1	91.3
fababean	11.92	83.3	0.71	1	59.9
field pea	7.74	87.3	0.61	1	47.6
lentil	8.47	85.4	0.54	1	98.2
kidney bean	8.47	84.2	0.51	2	0
mung bean	8.95	74.2	0.33	2	18.1
cowpea (black-eyed)	5.24	79.6	0.25	2	18.7
cowpea (brown)	3.24	78.3	0.11	2	19.3

(a) Dry weight basis.
(b) The yield of dehulled grain when 90% of the hull has been removed from the seed.
(c) DE = hull removed (g/100 g seed)/(100 – yield) (g/100 g seed).
(d) 1 designates loose adhesion between hull and cotyledon, whereas 2 denotes a tight binding.
(e) The weight percentage of seeds which have cotyledons bound together after dehulling.
Adapted from tables in Reichert *et al.* (1984).

For most commercial dehulling applications, abrasion or attrition mills are used, with attrition mills, e.g. plate mills, being favoured where the hull is less firmly attached to the seed coat. One problem with abrasion mills is that the yields are much lower than the theoretical yields and losses are higher because cotyledon material is lost with the hulls, sometimes as high as 30%. Soaking methods and residual mechanical hull removal are methods still widely used to evaluate the efficiency of hull removal. One problem with dehulling comes from the different size and shapes of the legumes, with dehullers designed specifically for each crop. There is some interest in a universal dehuller.

Reichert *et al.* (1984) also examined the dehulling performance of a multipurpose disc attrition mill with a variety of legumes. Their characteristics are compared in terms of yields and dehulling performance (see Table 9.11 footnotes for definitions).

Although there was little difference in the yields, which was the weight of dehulled product recovered when 90% of the hulls were removed, the dehulling performance, which gives the proportion of hulls in the abraded fines, gives a better indication of performance (see definition). Values ranged from 0.72 for soya bean down to 0.11 for brown cowpeas. Thus for soyabeans the abraded fines contains 72% hull materials, whereas for brown cowpeas it was only 11%, indicating big losses of cotyledon materials in the hulls.

Statistical analysis showed that the factors most responsible for differences in dehulling performance were seed hardness and resistance to splitting.

9.6.2 Peeling

Peeling is an important process for many processed and convenience fruit and vegetables. Mechanisms involved in peeling are abrasion, chemical cleaning, including caustic (lye) or brine and thermal peeling. Often more than one mechanism is involved and often spray washing is required to remove any loosely attached peel. All peeling operations generate solid waste and may cause damage to the material.

Abrasive peeling. The food is fed into a rotating bowl, which is lined with an abrasive material, such as carborundum. Rollers can also be used. The abrasion rubs off the skin, which is removed by water. Claimed advantages are (Fellows, 1990) low energy costs, minimal thermal damage, low capital costs. Drawbacks include higher product losses (up to 25%), production of large volumes of dilute wastes and relatively low throughputs. Some irregular shaped materials, for example potatoes with eyes, may need some manual inspection and finishing. Onion skins are easily removed by abrasion peeling. Knives may be used for citrus fruits.

Chemical peeling. A dilute solution of sodium hydroxide (1 to 2%) is heated to 100–120°C and contacted with the food for a short time period. Water sprays are then used to dislodge the skin. This was once popular for root vegetables, but it can cause some discoloration. It has now been largely replaced by steam peeling. The use of a more concentrated lye solution (10%) is known as dry caustic cleaning (Fellows, 1990) and reduces water consumption and produces a more concentrated waste for disposal. Brine solutions are also sometimes used.

Thermal peeling. The food is fed in batches into a pressure vessel, which rotates slowly. High-pressure steam is fed into the vessel and rapid heating occurs at the surface, within 15–30 s, but not in the bulk, due to the low thermal conductivity of the food, thereby minimising chemical reactions, including cooking, in the bulk of the food. The pressure is suddenly released, causing boiling of the liquid under the skin and flashing-off of the skin, which is removed with the condensed steam. Additional water sprays may be required. This method is increasing in popularity; it produces good quality products, with little damage, at high throughputs. There is minimum water utilisation and minimum losses. Flame peeling, using temperatures of 1000°C, has been used for onions.

9.6.3 Cleaning of raw materials

Contaminants on food raw materials can be of various origins:

- mineral – soil, stones, sand, metal, oil;
- plant – twigs, leaves, husks, skins;

- animal – faeces, hair, insects, eggs;
- chemical – pesticides, fertilisers, other contaminants;
- microbial – yeasts, moulds, bacteria and metabolic by-products, e.g. mycotoxins, e.g. patulin in apples.

One of the first preliminary operations must be to remove these. Important considerations are high efficiency of removal, combined with minimising loss and damage and further recontamination of components. A combination of methods is used, including dry cleaning and wet cleaning. Aspiration to remove dust and light contaminants has already been described.

Screening is widely used for removing contaminants considerably different in size to the food being treated. Sieves are available up to an aperture size of 25 mm and screens for larger sizes (see Section 9.3.2). Also disc separators, where the shape of the disc matches the shape of the food, can be used for separating seeds from grain.

It is important to be able to detect the presence of metal fragments and remove them from the raw material, to prevent damage to the food processing equipment and contamination of the final product. Metal detectors may also be incorporated at the end of the packaging line. Magnetic materials can be removed by powerful magnets, which can be permanent magnets or electromagnets. Non-ferrous metals, such as aluminium, are detected by passing the material through a strong electromagnetic field. This field is distorted and initiates a warning signal. X-rays have also been used for products in sealed containers. It has been reported that modern cocoa processing leads to iron contamination levels of 200 mg/kg in cocoa mass and greater than 300 mg/kg in cocoa powder; this arises from hammer mills, impact mills and the agitator blades of rotating ball mills. Between 5 and 15% of the metal was greater than 75 μm. Improvement in design of this equipment was considered to be the best way of reducing this (List and Thiede, 1987). Krishnan and Berlage (1984) looked at the principle of separating walnuts by a magnetic field. Iron dust with gelatin or a magnetic solution was added to the whole nut. This was cracked and the shell separated from the meat using a permanent magnet.

Electrostatic methods for cleaning materials are available, which take advantage of the differences in electrostatic charge of materials under controlled humidity conditions. The solid is fed from a hopper onto a drum, rotating at 70–350 r.p.m., which is either charged to a potential of 5–20 kV or earthed and the oppositely charged particles are separated as they are more strongly attracted to the drum. They are removed from the drum by a scraper. This method can be used to remove dust and stalk from tea fannings and also some unwanted seeds from cereals and oilseeds (Brennan *et al.*, 1990). The Dodder mill uses a roller coated with a velvet-type material, which will attract particles, such as seeds, and remove them from cereals, due to differences in their surface properties.

Wet methods are also widely used for cleaning purposes. Heavily soiled vegetables can be simply presoaked in water; the process helped by agitation. A more efficient process is spray washing, which uses high-pressure sprays and requires smaller volumes of water. The principles of flotation are used for cleaning vegetables; heavy particles such as soil, metal or glass sink, the vegetables are neutrally buoyant and straw and grass float. Screens may also be incorporated to remove oversize material. In wet-processing

applications, the microbial quality of water supply and the additional costs of the water and effluent treatment need to be considered.

Peeling or dehulling, as well as removing the outer layers, will also remove any disorders associated with them.

9.6.4 Sorting and grading

Sorting and grading are important preliminary operations. Sorting is normally reserved for processes which separate foods into categories based on a single physical property, such as size, shape, weight or colour. Grading, on the other hand, is a quality separation and a number of factors may have to be assessed. Some examples are colour, absence of blemishes, flavour and texture. Food grading is usually done manually, by trained experts, because it is not usually possible to link quality with one physical property.

Some examples are meat grading and inspection, fish grading, horticultural products, tea and cheese. However, the food analyst is always seeking for instrumental techniques for assessment of these sensory attributes which contribute towards the character of the food. For example the dielectric properties of fish have been found to change as the fish becomes less fresh. Consequently a wide range of instrumental methods has been evaluated for measurement both on-line and in the laboratory, of properties of foods that correlate with the sensory characteristics of appearance, colour, flavour and texture. Appropriate instrumentation and sensors have been reviewed by Kress-Rogers (1993).

Equipment for size sorting based on rollers and screens, which provide either a fixed or a variable size aperture, are discussed in more detail by Fellows (1990) and Brennan *et al.* (1990). Sorting by weight is important for high value products such as eggs, and some tropical fruits. Image analysis is being increasingly investigated in this respect.

Colour sorting and grading

Foods can be sorted on the basis of their colour, for example removing discoloured baked beans, prior to them being blanched. One of the most common applications is to pick out miscoloured pieces and the simplest method is by manual inspection, as the food passes by the inspectors, on conveyor belts.

Colour sorters have been available for over 40 years and one widely used application range is for particles in the range 2–10 mm. Some examples are: rice, baked beans and other legumes, peanuts and roasted coffee beans. Throughputs range from about 10 kg h^{-1} up to 10 000 kg h^{-1}, with many applications between 100 and 1000 kg h^{-1}.

These are based on a sensor located above the conveyor. The feed is divided into lanes or channels. Mohsenin (1984) summarises the sequence of operations as singularisation into discrete units, acceleration to present a substantial number of units to the system per unit time, presentation of each unit before the sensors, evaluation and comparison to some predetermined standard, and segregation to separate each unit according to its colour or other specified standard. Figure 9.12 illustrates the layout of such a sorter.

Colour sorting relies on the optical properties and reflectance of the samples. The principle is that the light source is directed on the material and the reflected light is measured by a photodetector and compared with preset standards. Materials outside the range are rejected. The incident light and reflected light may or may not be filtered, to

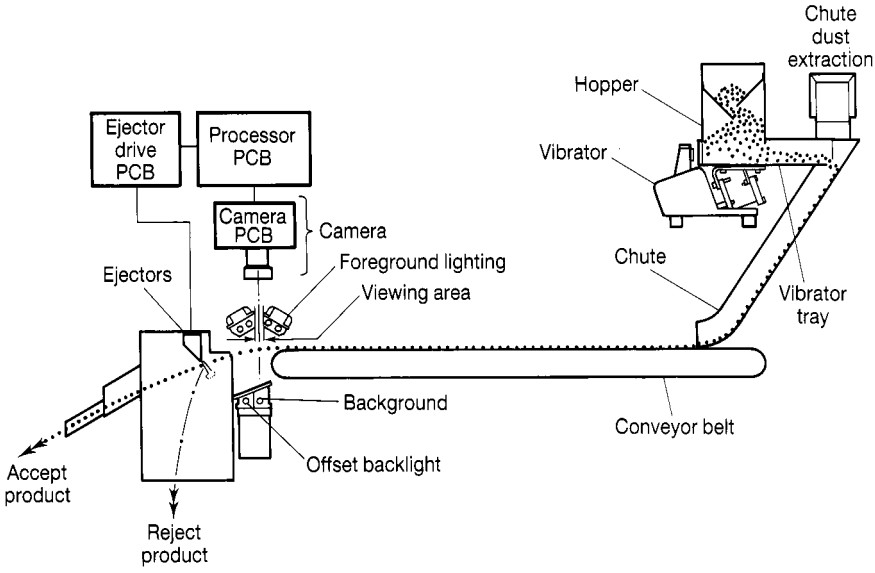


Fig. 9.12. Diagram of colour sorter (with courtesy of Sortex Ltd).

allow only selected wavelengths to reach the detector. Monochromatic sorting uses only one selected waveband range and is used where there is sufficient difference between the reflectivity of acceptable and unacceptable products within the selected waveband. Unfortunately it is not always possible to find a single section of the spectrum where this is the case, so it is necessary to resort to a more complicated procedure which involves measuring at two selected wavebands. This is known as bichromatic sorting. Usually a ratio of the signals from the two wavebands will facilitate sorting of the materials. In some cases dual monochromatic sorting is used, where it might be important to reject more than one type of defect. A discrepancy in either signal will cause the item to be rejected. More detail is provided by Low and Maughan (1993). Other factors to be considered are the natural variations in colour that are found for each product. The feed rate is also important and there may be problems operating in a dusty or humid environment.

As in most applications, such machines are never 100% efficient in terms of either removing all defective items or rejecting acceptable items. Improving the sensitivity of the detection unit increases the efficiency for removing defective items, but also means that a greater proportion of acceptable items will be rejected. There is also a recommended flow rate range for each machine. Increasing the flow rate within this range usually leads to a greater loss of acceptable material. In most cases, the overall performance improves if the material has been cleaned and size graded prior to colour sorting. Some typical examples of removal efficiencies for defective items are

- green coffee: removing defective beans and foreign material, 90% at 900 kg h^{-1};
- white beans: removing discoloured beans and foreign material, 97% at 1500 kg h^{-1};
- frozen peas: removing foreign material, 99% at 10 000 kg h^{-1}; (Low and Maughan, 1993).

Other applications are for sorting of fruit, picking out bruised, damaged or mouldy fruit. Colour measurement is used for control purposes; for example controlling the energy input into baking ovens to ensure a product of uniform colour from the oven. Transmittance methods form the basis of egg inspection and have been used to distinguish between cherries with and without pips.

Future developments will combine colour sorting with vision analysis, whereby sorting will be based on colour differences, size and shape.

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Index

adsorption recycle extraction, continuous, 196
adsorption systems, expanded bed, 195
adsorption, SCF, 40
affinity chromatography, 202
affinity separations, 189
air classification, 260
air classification, applications, 268
air classification, process characterisation, 264
air classifiers, equipment, 262
air jet sieve, 260
animal products, UF, 127
aqueous two-phase extraction, 182

backflushing, 146
bacterial removal, MF, 149
Beer, MF, 148
bioreactors, membranes, 128
biotechnology, recovery, UF, 133
blinding, ion exchange, 159
blood, UF, 127
bulk density, 252

capacity, ion exchange, 159
cell harvesting, MF, 150
centrifugation, 10
cereals processing wastes, RO, 91
cereals separations, AC, 268
chemical properties, SCF, 23
cholesterol lowering, SCF, 52
cider, MF, 148
clarification, MF, 148
cleaning, membranes, 84
cleaning, solids separations, 279
coffee, decaffeination, 49
coffee processing, RO, 91
colour sorting and grading, 281
columns, IE, 160
composition, food, 1
concentration factor, 69
concentration polarisation, 72
concentration polarisation, UF, 106, 146
critical T and P for supercritical fluids, 20
crystallisation, 230
crystallisation, fats, 209
cut size, 264

dairy applications, UF, 121
decaffeination, tea and coffee, 49
decolorisation, IE, 163
dehulling, 278
dehydration, 9, 12
demineralisation, IE, 161
density, bulk, 252
density, particle, 250
density, supercritical fluids, 20
detergent fractionation, 230
diafiltration, 116
diafiltration, applications, 119
diafiltration, washing in, 118
diafiltration, washing out, 117
diffusion coefficients, 34, 39
diffusion, supercritical fluid, 22
dry fractionation, 211

eggs, RO, 92
eggs, UF, 128
electrically enhanced separations, 202
electrodialysis, 166
electrodialysis applications, 169
electrodialysis equipment, 167
electrophoresis, 202
electrostatic cleaning, 280
elution, ion exchange, 159
emulsion, liquid membranes, 193
energy utilisation, UF, 114
entrainers, 34
enzyme purification, IE, 165
enzyme purification, LLE, 182

enzyme recovery, MF, 151
equations of state, 31
equilibrium, 6
equipment, fat fractionation, 211
equipment, RO and UF, 75
equipment, SCF, 42
evaporation, 12
expanded bed adsorption, 195
extraction, biochemicals, 4
extraction, mechanisms, SCF, 37

fat crystallisation, 209
fat fractionation, 207
fat fractionation, membrane filters, 221
fermenters, membranes, 131
filtration, 10
filtration equipment, fats, 212
filtration performance, 216, 218
fish, UF, 128
flash cooling, 12
flavour extraction, SCF, list of substances, 56
flavour extraction, SCF, 54
flavour extraction, spinning cone, 14
Florentine filter, 212
flowability, powders, 254
food composition, 1
food composition, table, 2
forces of adhesion, 252
formed-in-place membranes, 92
fouling, ion exchange, 159
fouling, MF, 146
fouling, UF, 111
fractionation, SCF, 47
fractionation, SCF, butterfat, 53
fractionation, SCF, fish oils, 53
free diffusion model, 37
fruit juices, MF, 149
fugacity, 29

grade efficiency, AC, 266
grading, colour, 282
grading, solids, 281

Hausner ratio, 253
hollow fibre, 77
hops, 54
hydrofilter press, fat fractionation, 227
hydrophobicity, 183
hygiene, membranes, 82

innovative methods, introduction, 179
innovative processing, solid-based systems, 195
inorganic membranes, 142
intracellular proteins, purification, 203
ion exchange applications, biotechnology, 165, 166
ion exchange, applications, food, 160
ion exchange, capacity, 159
ion exchange, equipment, 160
ion exchange, fermentation broths, 165
ion exchange, materials, 155, 158
ion exchange, proteins, 163
ion exchange, theory, 155

Jenike flow cell, 255

kinetics, innovative methods, 181

lecithin purification, SCF, 52
legumes, dehulling, 278
legumes, separations, AC, 270
Lipofrac fractionation process, 234
liquid–liquid extraction, 181
liquid membrane separations, 191
low-alcohol drinks, RO, 92

mass transfer, innovative methods, 181
materials, chromatographic and adsorption, 201
medical applications, UF, 134
membrane bioreactors, 128
membrane characteristics, 70
membrane chromatography, 199
membrane configuration, 76, 142
membrane configuration, MF, 142
membrane fermenters, 131
membrane filters, fat fractionation, 221
membrane terminology, 66
membranes, downstream processing, 133
membranes, inorganic, 142, 145
membranes, introduction, 65
membranes, liquid, 191
membranes, sharp, diffuse, 100
metal detection, 280
microfiltration, bacterial removal, 149
microfiltration, beer, cider, 148
microfiltration, equipment, 142
microfiltration, fouling, 146
microfiltration, fruit juices, 149
microfiltration, introduction, 141
microfiltration, milk, 149
microfiltration, performance, 146
microfiltration applications, biotechnology, 150
microfiltration applications, food, 148
milk, demineralisation, 161
milk, MF, 149
milk, UF, 121
milk, composition, 113
milk processing, RO, 88
milk fat filtration, 217
milk fat fractionation, 216
mixed bed systems, IE, 160

oil extraction, SCF, 50
oils, quality, 210
oilseed proteins, UF, 125

osmotic pressure, 68
osmotic pressure values, 68
other applications, AC, 273

particle density, 250
particle shape, 250
particle size, 247
particle size distribution, 247
partition coefficient, 184
partitioning, factors affecting, 183
peeling, 279
Peng Robinson equation, 31
perfluorocarbon affinity separation, 189
perfusion chromatography, 201
permeate flux, UF, 106
permeate flux, factors affecting, 114
permeate rate, 71
physical properties, 5
physical properties, solids, 244
physicochemical basis for separations, 180
plate and frame modules, 79
polymorphism, fats, 210
porosity, 252
powders, bulk properties, 252
powders, methods of classification, 246
powders, quality factors, 246
protein fractionation, UF, 119
protein purification, IE, 163
protein recovery, IE, 163
protein recovery, wet separation, 274
protein shifts, air classification, 267
purification applications, IE, 166

raw materials, cleaning, 279
recombinant DNA techniques, 203
Redlich-Kwong equation, 31
rejection, 69, 98
rejection, average, 103
rejection, biochemicals, 99
rejection data, practical data, 104
rejection, UF, milk components, 99
retention, see rejection
reverse-micelle extraction, 185
reverse osmosis, fruit and vegetable juices, 90
reverse osmosis, other applications, 91
reverse osmosis, water treatment, 87
rotary drum filter, fat, 215

Safety aspects, *membranes*, 82
Sauter mean particle diameter, 249
superficial fluids, applications, 48
supercritical fluids, chemical properties, 23
separation, from solids, 7
separation, powders and particulates, 256
separation, SCF, 42
separations, from gases, 13
separations, from liquids, 11
separations, liquid–solid, 10

shrinking core model, 38
sieve sizes, 259
sieving, 258
size reduction, 256
softening, ion exchange, 161
solid separations, summary, 244
solids, dry separations, 258, 260
solids, separations from, 7
solids, wet separations, 273
solids separations, 243
solids separation, miscellaneous processes, 278
solubilities, SCF, 24
solvent fractionation, fats, 236
Sortex colour sorter, 282
sorting, colour, 281
sorting, solids, 281
soya concentrates and isolates, 274
soya processing, 274
spinning cone column, 14
spiral wound module, 79
stirred tanks, IE, 160
Stockdale filter, 220
Stokes equation, 264
sugar juice demineralisation, 162
supercritical extraction, introduction, 17
supercritical fluid state, phase diagram for CO_2, 19
supercritical fluids, density, 20
supercritical fluids, diffusion, 22
supercritical fluids, equipment, 42–47
supercritical fluids, theoretical models, 29
supercritical fluids, viscosity, 21
supercritical fluids, volatility, 23
supported liquid membrane, 192

tea, decaffeination, 49
transport phenomena, UF, 106
tubular modules, 76

ultrafiltration, animal products, 127
ultrafiltration, applications, 121
ultrafiltration, introduction, 97
ultrafiltration, medical applications, 134
ultrafiltration, oilseed proteins, 125
ultrafiltration, permeate flux, 106
ultrafiltration, vegetable proteins, 125
ultrafiltration fouling, 111

Vacuband batch filter, 214
van der Waals equation, 67
Vant'Hoff equation, 67
vegetable proteins, UF, 125
Virial equation, 67
viscosity, supercritical fluid, 21

water, in foods, 9
water, role in SCF extraction, 40
water, softening, 161
water treatment, 15

water treatment, RO, 87
wet separation processes, 273
wheat protein, wet separations, 276
whey composition, 113
whey, demineralisation, 161
whey, ED, 171
whey, protein recovery IE, 164
whey, UF, 123

yield, air classification, 266, 272
yield, UF, 101